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THE INFLUENCE OF IONIZING RADIATION ON THE THERMOPHYSICAL PROPERTIES OF MEAT FROM THE BROILER CHICKENS WITH DIFFERENT STRESS RESISTANCE

Roza T. Timakova[1], Sergey L. Tikhonov[1], Natal'ya V. Tikhonova[1], Sergey V. Shikhalev[1], Leonid S. Kudryashov[2*]
[1]Ural State Economic University, Ekaterinburg, Russia
[2]V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia

Keywords: *stress-resistant and stress-sensitive broiler chickens, ionizing radiation, thermophysical properties*

Abstract

The studies on the influence of radiation treatment of carcasses from the stress-resistant and stress-sensitive broiler chickens on the thermophysical properties of raw meat are presented. An increase in thermal diffusivity of meat from the stress-resistant poultry by 24.7 % and 54.7 % after radiation treatment of carcasses with ionizing radiation doses of 9 kGy and 12 kGy, respectively, was established. In meat from the stress-sensitive poultry, this figure increased by 33.3 % and 35.8 % compared to the untreated carcasses.

It is shown that radiation treatment of carcasses by applied doses increased the thermal conductivity coefficient of meat from the stress-resistant poultry by 5.3% and 7.0 %; in meat from the stress-sensitive poultry, this figure increased by 2.0 and 6.2 times compared to meat from the carcasses not exposed to radiation. At the same time, the value of the heat capacity coefficient was reduced.

The irradiated poultry meat samples accumulate energy of ionizing radiation more intensively, which allows the intensification of the thermal processes occurring at various stages of meat product production. Treatment of meat from the stress-resistant poultry with ionizing radiation can reduce the amount of meat with non-traditional autolysis due to changes in its functional-technological properties. The results of the research should be taken into account in technological processes in the production of meat products with non-traditional autolysis.

Introduction

The task of maximum preservation of food raw materials and food products at all stages of their production and storage takes on great significance. Meat and meat products are perishable produce. Therefore, it is necessary to chill them as soon as possible to minimize the growth of bacterial pathogens and store them at a low temperature to ensure microbiological safety.

The methods of poultry carcass chilling (water or air) and thermophysical properties of meat raw materials affect the post mortem changes in meat and, therefore, the quality of manufactured products. High rates of carcass chilling at the initial stage of the technological cycle can cause the so-called cold shortening of muscles; as a consequence, meat becomes tough during the whole period of post mortem storage. Fast chilling of carcasses can facilitate occurrence of meat with non-traditional properties, in particular, DFD meat. To improve the functional-technological properties of meat with DFD and PSE defects, manufacturers use different phosphate containing additives, salts of organic acids, different methods of meat chilling, electrical stimulation and so on [1,2,3,4,5].

According to the data of several authors [6], the physical parameters of meat such as the thermal conductivity coefficient, thermal diffusivity and specific heat are influenced by stress resistance of animals and poultry. It is associated with the fact that animals and poultry with different stress resistance are characterized by the different ratio of the muscle and fatty tissues, ratio of free and bound moisture, which have different thermophysical properties.

It is known that DFD meat is unstable in storage due to the high pH value and possible rapid contamination by microorganisms.

One of the promising methods for increasing shelf-life of meat raw materials, including meat with DFD properties can be its treatment with ionizing radiation according to GOST 33820–2016 "Fresh and frozen red meat. Guidance for irradiation to control parasites, pathogens and other microorganisms" and GOST 33825–2016 "Packed semi-finished meat. Guidance for irradiation to control parasites, pathogens and other microorganisms". The use of radiation treatment can significantly increase meat shelf life at low energy and material expenses compared to traditional methods [7,8,9,10]. Treatment with ionizing radiation of initial meat raw materials ensures microbiological stability and allows increasing shelf life of vacuum-packed meat semi-finished products by more than 3 times [11,12]. Moreover, meat treatment with ionizing radiation has a positive effect on ageing process. The mechanism of action of ionizing radiation on the biochemical processes of meat

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ageing can be presented as follows. According to the law of conservation of energy "Energy does not disappear and is not created, but transforms from one form into another". The energy of ionizing radiation is absorbed by the muscle tissue, which contains a significant amount of water with dissolved proteins, nucleic acids, enzymes, hormones and other biologically active substances, which leads to its radiolysis (a process of radiation destruction) [13] and correspondingly, to the intensive destruction of protein substances [14] both under the action of radiation per se and by the action of the radiation-activated tissue proteolytic enzymes, to which the energy that was initially absorbed by water is transferred. One of the main conditions for manifestation of the activity of the radiation-activated tissue proteolytic enzymes throughout a product volume is a uniform temperature of the muscle tissue, which determines the thermophysical characteristics of meat. The ionizing radiation is one of the means of heat (energy) transfer, and radiation treatment of meat allows the even distribution of heat obtained by the muscle tissue ensuring the same activity of tissue enzymes in all layers of the muscle tissue, which is important for reducing meat ageing duration and preventing uncharacteristic changes of the muscle tissue in the process of autolysis.

The above-mentioned hypothesis can be explained by the ability of ionizing radiation to activate proteolytic reactions, occurring in the muscle tissue under the effect of cathepsins and calpains, which leads to an increase in the number of free carboxyl and amino groups in the protein molecule, and increases hydration and tenderness of meat. Swelling of connective tissue collagen and muscle tissue proteins increases an amount of bound moisture, correspondingly, influences the thermal conductivity coefficient of meat raw materials [15].

In this connection, the aim of this work is to study an effect of different doses of ionizing radiation on the thermophysical properties of meat from broiler chickens with different stress resistance.

Objects and methods

The objects of the research were the meat samples from broiler chickens, which were chilled to an internal temperature in muscles of -2 °C to 4 °C inclusively (according to GOST 31962–2013 "Chicken meat (carcasses of chickens, broiler-chickens and their parts"). The meat samples were taken from male Arbor Acres broiler chickens (42-day-old) raised in the Bektysh poultry farm and divided into two groups by phenotypical traits using the method of balanced analogous groups: stress-resistant chickens (SRC) and stress-sensitive chickens (SSC). Stress resistance of broiler chickens was determined by the method described in [16]. Poultry keeping and feeding corresponded to the zoohygienic requirements and recommendations of the All-Russian Research and Technological Institute of Poultry upon cage technology of chicken keeping.

For the experiment, six groups of meat were formed: group 1 (control) – the meat samples from the stress-resistant birds (not treated with ionizing radiation); group 2 (experimental) – the meat samples from the stress-resistant birds treated with a dose of 9 kGy; group 3 (experimental) – the meat samples from the stress-resistant birds treated with a dose of 12 kGy; group 4 (control) – the meat samples from the stress-sensitive birds (not treated with ionizing radiation); group 5 (experimental) – the meat samples from the stress-sensitive birds treated with a dose of 9 kGy; group 6 (experimental) – the meat samples from the stress-sensitive birds treated with a dose of 12 kGy.

Ionizing radiation treatment was carried out in the Center of radiation sterilization of the Ural Federal University named after B.N. Yeltsin on the electron linear accelerator (UELR-10-10C2 model) with the energy of up to 10 MeV at doses of 9 and 12 kGy. The experiments were carried out with five replications.

To determine the thermophysical characteristics based on the thermal conductivity coefficient, thermal diffusivity and specific heat coefficient, the method of the regular regime of chilling by G.M. Kondratiev was used [17].

Boneless meat tissue of broiler chickens was sampled by GOST R "Meat and meat products. Methods of primary sampling". During the experiment, poultry meat samples were minced to forcemeat condition according to the requirements of GOST R 55365–2012 "Minced meat. Specifications."

Experimental investigations were carried out on the pilot unit [16] (Figure 1) consisted of the α-calorimeter 7 in a form of a spherical copper jacket with minced poultry meat under investigation, thermostat 1 with a lid 2, agitator with a drive 6, chromel-copel thermocouples 3 to detect a temperature in samples and refrigerating (water/air) media, potentiometer 4 of the precision class 0.25 with incubation of the free ends of the thermocouples and the control thermocouple.

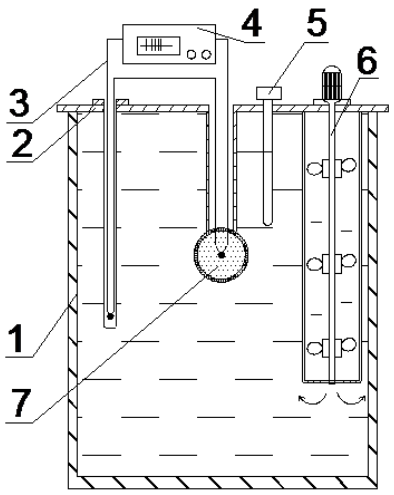

Figure 1. Schematic diagram of the pilot unit: 1 - thermostat; 2 - lid; 3 - differential thermocouple; 4 - potentiometer; 5 - thermometer; 6 - agitator; 7 - calorimeter

Figure 2 presents a schematic layout of the spherical calorimeter used in the pilot unit.

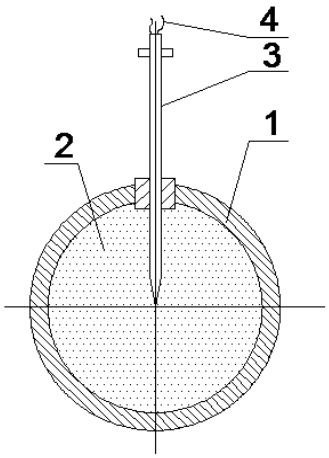

Figure 2. Spherical calorimeter: 1 - calorimeter jacket; 2 – product under investigation; 3 – protective tube; 4 - thermocouple

After heating and incubation of the calorimeter to a temperature that did not exceed the temperature of denaturation of thermally labile meat muscle proteins, it was chilled in a liquid thermostat at an intensive agitation using an agitator; that is, the conditions were created under which thermal diffusivity from the calorimeter surface to the refrigerating medium had high values close to [17]. In this case, thermal diffusivity can be determined from the transcendent equation for a sphere

$$a = m_{\infty}\left(\frac{R}{\pi}\right)^2, \qquad (1)$$

where R – is the radius of the calorimeter (copper sphere);
m_{∞} - the chilling rate.

The m_{∞} value was determined on the basis of the graphic dependence of the excess temperature in the semi-logarithmic coordinates.

Determination of the thermal conductivity coefficient was carried out on the stand unit (Figure 3) consisted of the λ-calorimeter 5 in a form of a spherical copper jacket with minced poultry meat under investigation, air-bath thermostat 4 with a fan 3, chromel-copel thermocouples 1 and potentiometer 2 of the precision class ±0.25% with incubation of the free ends of the thermocouples.

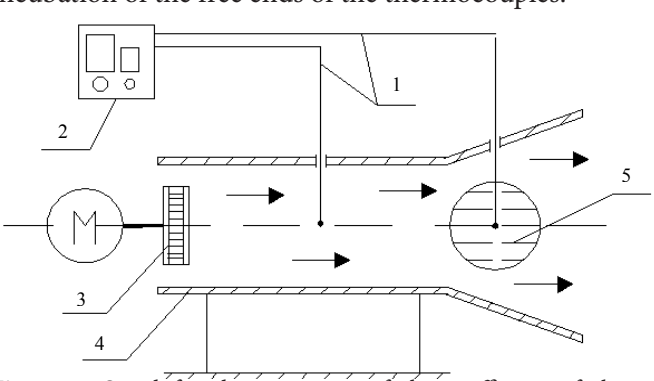

Figure 3. Stand for determination of the coefficient of thermal conductivity: 1-chromel-copel thermocouples; 2- potentiometer; 3- fan; 4- air-bath thermostat; 5-λ- calorimeter

In this case, the thermal conductivity coefficient is determined by the characteristic equation for a sphere:

$$\lambda = \frac{\bar{\alpha}\cdot R}{1 - R\cdot\sqrt{\frac{m}{a}}\cdot \operatorname{ctg}\left(R\sqrt{\frac{m}{a}}\right)}, \qquad (2)$$

where m – the chilling rate at these conditions, determined by the above-mentioned method;

$\bar{\alpha}$ - thermal diffusivity, determined on the reference calorimeter by the method of comparison with a sample having the known thermal conductivity coefficient.

To determine the specific heat capacity, the method of the comparative chilling of the control and experimental samples of minced poultry meat was used, where the flour of the higher grade with the known thermal conductivity coefficient was used as a reference by obtaining the chilling curves in a time interval. Calculations were made by the equation:

$$\tilde{n}_m \frac{\partial t_m}{\partial \tau} = \tilde{n}_r \frac{\partial t_r}{\partial \tau}, \qquad (3)$$

where c_m, c_r – the coefficients of the specific heat capacity of the sample (poultry meat) and reference (flour);

t_m, t_r – a temperature of the sample (poultry meat) and reference.

As the experiments showed, thermal diffusivity of minced poultry meat increased when the temperature decreased, which is consistent with the published data [18].

The statistical processing of the research results was carried out using the standard computer programs Microsoft Excel XP, Statistica 8,0.

Results and discussion

The results of the performed investigations are graphically presented in Figure 4 and Figure 5, and in Table 1.

It was established that upon minced meat chilling in a liquid and air-bath thermostat, the chilling rate of the meat samples from the stress-sensitive birds of the 4th (control), 5th (experimental) and 6th (experimental) groups was lower than in the samples from the stress-resistant birds (1st, 2nd and 3rd groups) (Figure 4 and Figure 5). As the obtained results indicate, minced meat treatment with ionizing radiation at doses of 9 and 12 kGy allows increasing thermal diffusivity of minced meat from the stress-resistant birds by 24.7 % and 54.7 %, and from the stress-sensitive birds by 33.3 % and 35.8 %, respectively.

It is necessary to note that in the irradiated samples from the stress-sensitive birds, the values of thermal diffusivity increased to the levels that corresponded to the non-irradiated minced meat from the stress-resistant birds. For example, in the samples from the stress-sensitive birds of the 5th and 6th experimental groups with the irradiation doses of 9 kGy and 12 kGy, thermal diffusivity reached and exceeded the value of this indicator in the non-irradiated stress-resistant birds from the 1st group.

Table 1. Changes in the thermophysical properties of meat from birds with different stress resistance upon processing with ionized radiation (n=5)

Thermophysical parameter	Stress-resistant poultry			Stress-sensitive poultry		
	Without treatment (group1)	Irradiation dose 9 kGy (group 2)	Irradiation dose 12kGy (group 3)	Without treatment (group 4)	Irradiation dose 9 kGy (group 5)	Irradiation dose 12kGy (group 6)
Thermal diffusivity, m^2/s	$7.3 \cdot 10^{-8}$	$9.1 \cdot 10^{-8}$	$11.29 \cdot 10^{-8}$	$5.89 \cdot 10^{-8}$	$7.85 \cdot 10^{-8}$	$8.0 \cdot 10^{-8}$
Thermal conductivity coefficient, W/(m•K)	0.57	0.60	0.61	0.098	0.20	0.306
Specific heat coefficient, J /(kg•K)	5321	4670	4317	4358	4843	3995

*reference values of minced meat from broiler chickens ā=10.9•10-8m2/s. λ=0.41W/(m•K), cp = 3559 J/(kg•K) [17].

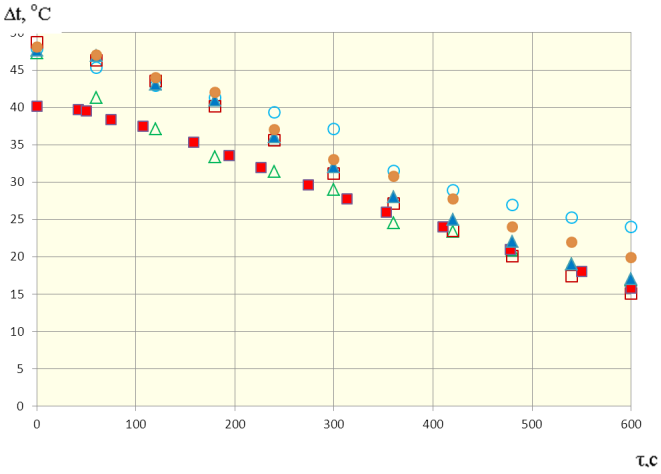

Figure 4. Chilling rate of the poultry minced meat samples in the liquid thermostat : ●– control group 1 (SRC); ▲ – experimental group 2 (SRC) (9 kGy); □– experimental group 3 (SRC) 3 (12 kGy); ○– control group 4 (SSC); Δ – experimental group 5 (SSC) (9 kGy); ▪– experimental group 6 (SSC) (12 kGy) Spherical calorimeter: 1 - calorimeter jacket; 2 – product under investigation; 3 – protective tube; 4 - thermocouple

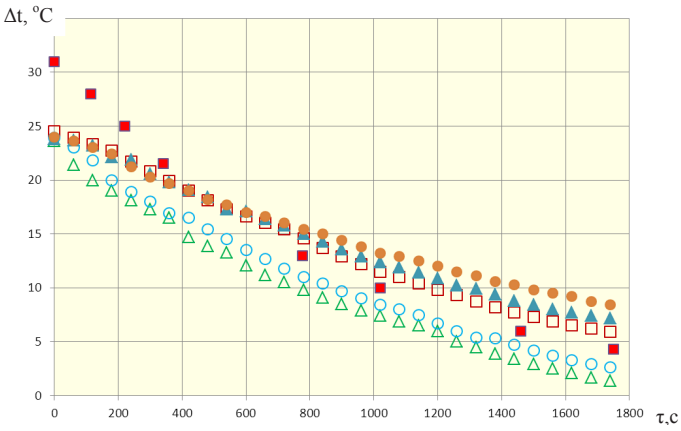

Figure 5. Chilling rate of the poultry minced meat samples in the air-bath thermostat: ●– control group 1 (SRC); ▲ – experimental group 2 (SRC) (9 kGy); □– experimental group 3 (SRC) (12 kGy); ○– control group 4 (SSC); Δ – experimental group 5 (SSC) (9 kGy); ▪– experimental group 6 (SSC) (12 kGy)

It is possibly associated with the fact that after exposure of meat to ionizing radiation, the chain reactions of ionization are activated and excitation of atoms occurs with the development of different products of water radiolysis: hydroxyl radicals, hydrated electrons and hydrogen atoms having the high reaction capacity. According to the available information [19,20], the observed change in the moisture binding capacity and moisture content in the studied samples of minced meat is a key factor of the changes in the thermal diffusivity value.

Analysis of the experimental values of the thermal conductivity coefficient showed that meat from the stress-resistant birds was characterized by the high ability to conduct the amount of heat. Moreover, as a result of irradiation of the meat samples from the stress-resistant broiler chickens with a dose of 12 kGy, the value of the thermal conductivity coefficient increased by 7.0 % compared to the non-treated samples, in meat from the stress-sensitive birds by 6.2 times, respectively. As can be seen from table 1, ionizing radiation had the most significant effect on the value of the thermal conductivity coefficient of meat from the stress-sensitive birds. In our opinion, an increase in λ could be influenced by the total fat content in the muscle tissue, which thermal conductivity is three times lower than thermal conductivity of the meat muscle tissue [21]. For example, it was found in [16] that the stress- sensitive broiler chickens had somewhat larger depositions of subcutaneous fat compared to the stress-resistant birds.

The specific heat coefficients of the meat samples from the stress-sensitive birds had lower values compared to the corresponding samples from the stress- resistant birds. The value of the specific heat coefficient of the meat samples from groups 4, 5 and 6 was 22.1 %, 3.7 % and 8.1 % lower compared to the samples of the stress-resistant birds from groups 1, 2 and 3, respectively. With that, a decrease in the specific heat coefficient was observed practically in all six groups of the meat samples depending on a dose of ionizing radiation, which can be explained by a decrease in the humidity and fat content in irradiated poultry meat [21].

After meat treatment with ionizing radiation, a positive effect on the organoleptic indicators was noticed. For example, the consistency of the meat samples from the stress-sensitive broiler chickens became firmer, their moisture content decreased, correspondingly, the signs of meat with uncharacteristic autolysis (PSE) were leveled.

Conclusions

Therefore, as the results of the investigations showed, irradiation differently affected the change in the thermophysical properties of meat from the stress-resistant and stress-sensitive broiler chickens. After treatment with ionizing radiation with doses of 9 kGy and 12 kGy, it was found that thermal diffusivity increased by 24.7 % and 54.7 % in the meat samples from the stress-resistant birds and by 33.3 % and 35.8 % in the meat samples from the stress-sensitive birds, respectively, compared to the untreated poultry meat samples.

An increase in the coefficients of heat conductivity by 5.3 % and 7.0 % was found in meat from the stress-resistant birds and by 2.0 and 6.2 times in meat from the stress-sensitive birds depending on the dose of irradiation upon a decrease in the value of the of heat capacity coefficient.

The irradiated meat samples more intensively accumulate the energy of ionizing radiation, which allows intensification of thermal processes occurred at different technological stages of meat product manufacture. Processing of meat from stress-sensitive birds with ionizing radiation allows decreasing quantity of meat with non-traditional autolysis due to the changes in its functional and technological properties. It is necessary to take into account the obtained results of the investigations in the technological processes when manufacturing products from meat with uncharacteristic autolysis as well as in automation of processes of chilling and thermal processing of minced meat, smoked products and other meat products from irradiated raw materials.

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AUTHOR INFORMATION

Roza T. Timakova – candidate of agricultural Sciences, docent, Department of tourism and hospitality, Institute of trade, food technologies and service, Ural state University of Economics, 620144, Ekaterinburg, 8-th Marta Str./Narodnoy voli str., 62/45. Tel.:+7-912-247-99-74. E-mail: trt64@mail.ru
Sergey L. Tikhonov – doctor of technical Sciences, Professor, head of Department "Food engineering", Institute of trade, food technologies and service, Ural state University of Economics, 620144, Ekaterinburg, 8-th Marta str./Narodnoy voli str., 62/45. Tel.:+7-912-227-69-89. E-mail: tihonov75@bk.ru
Natal'ya V. Tikhonova – doctor of technical Sciences, docent, Department "Food engineering", Institute of trade, food technologies and service, Ural state University of Economics, 620144, Ekaterinburg, 8-th Marta str./Narodnoy voli str., 62/45. Tel.:+7-912-227-69-89. E-mail: tihonov75@bk.ru
Sergey V. Shikhalev– candidate of technical sciences, docent, Department "Food engineering", Institute of trade, food technologies and service, Ural state University of Economics, 620144, Ekaterinburg, 8-th Marta str./Narodnoy voli str., 62/45 Tel.:+7-922-613-11-09. E-mail: sershih@rambler.ru
Leonid S. Kudryashov – doctor of technical sciences, professor, chief researcher, V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, 109316, Moscow, Talalikhina str., 26. Tel.: +7-903-627-33-06. E-mail: lskudryashov@yandex.ru. *corresponding author

Contribution

Authors equally contributed to the writing of the manuscript and are equally responsible for plagiarism

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Original scientific paper

CLIMATE CHANGE: IMPACT ON MYCOTOXINS INCIDENCE AND FOOD SAFETY

Dragan Milicevic*, Brankica Lakicevic, Radivoj Petronijevic, Zoran Petrovic, Jelena Jovanovic, Srdjan Stefanovic, Sasa Jankovic

Institute of Meat Hygiene and Technology, Belgrade, Serbia

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Abstract

Climate change may have an impact on the occurrence of food safety hazards along the entire agri-food chain, from farm to fork. The interactions between environmental factors and food contamination, food safety and foodborne diseases are very complex, dynamic and difficult to predict. Extreme weather conditions such as floods and droughts which have not occurred previously in Serbia, may be supporting factors to contamination of crops by various species of toxigenic fungi and related mycotoxins. Mycotoxins are a group of naturally occurring toxic chemical substances, produced mainly by microscopic filamentous fungal species that commonly grow on a number of crops and that cause adverse health effects when consumed by humans and animals. Recent drought and then flooding confirmed that Serbia is one of the few European countries with very high risk exposure to natural hazards, as well as that mycotoxins are one of the foodborne hazards most susceptible to climate change.

Introduction

Serbia is one of the leading agriculture producing countries in the region, which has made agricultural production traditionally an important part of the national economy. According to the Statistical Office of the Republic of Serbia [1], the agriculture accounts for about 15,9 % of Gross Domestic Product (GDP) in - 3rdQuarter 2018. Climate change is a current global concern due to the continuing controversy about the magnitude of its effects on the food production systems and food supply chain. The impact of climate change on agriculture is particularly high in undeveloped and developing countries such as Serbia, due to the difficult economic situation and small investments in the improvement, particularly of primary production. The FAO/UN [2] Convention on Climate Change defines climate change as "a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods." The impacts of climate change on food, environmental and agriculture are interrelated across public health, social and economic dimensions. Therefore, the Convention defines adverse effects of climate change as changes that have deleterious effects on ecosystems, socio-economic systems, and human and animal welfare. One of the main objectives of the Convention is to predict how climate change could affect food safety and quality and to establish generic model risk management policies for mycotoxins contamination, because all of the factors involved in climate change will vary depending on the region or the country. This study review: 1) the potential impacts of predicted changes in climate on mycotoxins contamination 2) to discuss their implications on the food supply chain and possible consequences for public health and finally to 3) identifies adaptation strategies and research priorities to address food safety implications of climate change from the Serbian perspective.

An overview of the country profile

Serbia is a country situated at the crossroads between Central and Southern Europe on the Balkan Peninsula. In 2017 the total population was 7 million. The climate of Serbia can be classified as a warm-humid continental or humid subtropical climate, with more or less pronounced local characteristics (Figure 1). The hottest month is July with a apsolulte maximum air temperature ranging from 37.1 to 42.3 °C. Most of Serbia has a continental rainfall regime, with larger quantities in the warmer half of the year, except in the southwestern regions where the highest precipitation is measured in autumn. The worst is June, when an average of 12 % to 13 % of the total annual rainfall drops on average. The least precipitation occurs in February and October [3]. The country's economy is dominated by the industrial sector and agriculture. Serbia is one of the main grain producers and exporters in Europe (wheat, maize) [4].

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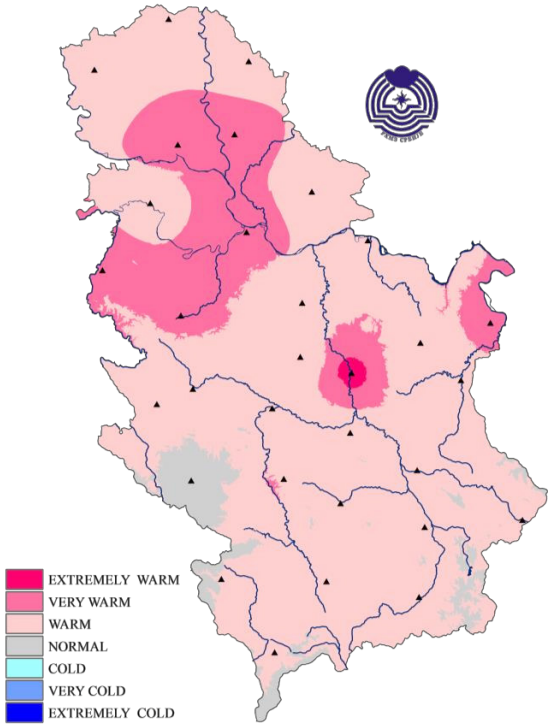

Figure 1. Spatial distribution of the mean annual air temperature based on the percentile method [3]

Food control management

Depending on the purpose, food control management could be defined as "the mandatory regulatory activity of the enforcement of food laws and regulations by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing and distribution are safe, wholesome and fit for human consumption; conform to safety and quality requirements; and are honestly and accurately labelled as prescribed by law" [5]. In addition to this, food control managementhas been defined as "a continuous process of planning, organizing, monitoring, coordinating and communicating, in an integrated way, a broad range of risk-based decisions and actions to ensure the safety and quality of domestically produced, imported and exported food for national consumers and export markets as appropriate" According to Codex Alimentarius Commission [6] working principles food safety should be based on risk analysis with an integrated-longitudinale from farm to table approach. In Serbia, competences for food and feed safety, animal health, animal welfare and plant health are assigned at national level to Ministries and their related agencies. The Ministry of Agriculture is the leading ministry involved in the food chain. Within the Ministry, Veterinary Directorate, Plant Protection Administration and Directorate for National Reference Laboratories has overall responsibility for food and feed safety. The Ministry's of Health (Sector for Inspection) role, in the context of the food safety system, is limited and responsible for the official controls of food contact materials, food supplements and food for particular nutritional uses.

Official food control laboratories

According to international agencies responsible for food safety and quality, laboratories are an essential component of a food control system and require considerable resources to set up, maintain and operate. Official food control laboratories are accredited by the Serbian accreditation body (ATS) according to ISO 17025, and participating in the inter-laboratory proficiency testing schemes. However, the regulatory laboratories are managed by different ministries, thus the lack of effective coordination among the different laboratories causes duplication in the work and a waste of resources. Harmonization of methods and techniques is one of the challenges in the official laboratories in Serbia, where each individual laboratory often follows its own analytical methods. However, food standards and limits published by the Institute for Standardization of Serbia are followed by most laboratories unless international requirements apply.

Impact of climate change factors in Serbia on growth and mycotoxin production

Over the last three decades a lot of works has been focused on the environmental impact on growth and mycotoxin production by a wide range of mycotoxigenic fungi. Most of them revealed that climate changes have a great impact on growth of many mycotoxigenic fungi. As mentioned, temperature and water activity (aw) is a primary determining factor that modulates fungal growth and mycotoxin production [7]. Therefore, drought is a modulator of mycotoxin contamination that is expected to be more frequent, depending on geography. Serbia has a continental to moderate continental climate. The country often has heavy rainfall. The temperatures (up to 40 °C) and relative humidity (up to 80 %) are high throughout the year. According to a report by the Republic Hydrometeorological Service of Serbia, climatic changes resulted in specific extreme conditions, which have not occurred previously in Serbia in the period from 2011 to 2013 and 2015 producion years. Prolonged periods of extremely high air temperatures during summer of 2012 (daily temperatures near 40 °C) (Figure 2), as well as precipitation deficit, resulted in highest average frequency of *Aspergillus spp.* particularly *A. flavus* and *A. niger* on the analyzed grain [8]. In addition, after high air temperatures during summer, heavy total rainfall was recorded during the winter of 2011/2012 and 2012/2013, thus in most of Serbia location precipitation was on the historical maximum. This consequently induced a high average moisture content in harvested maize kernels (>12 %), followed by *Fusarium* and *Penicillium* growth and production of related mycotoxins. Diseases produced by *Fusarium* toxins are one of the major threats to farmers in Serbia [9]. Under such climate conditions a high presence of mycotoxins can be expected, as well as the co-occurrence of multiple mycotoxins in cereals [10]. Since the presence of mycotoxins may potentially affect human and animal health, maximum levels (ML) have been established for

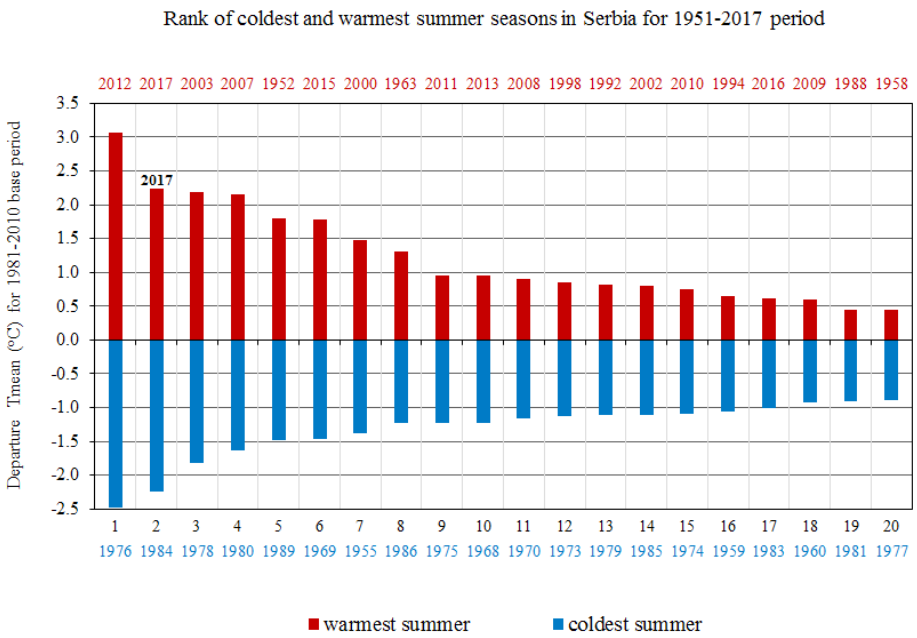

Figure 2. Rank of coldest and warmest summer seasons in Serbia for 1951-2017 period [3]

11 mycotoxins in food: AFTs (the sum of AFB1, B2, G1, and G2) as well as AFB1 alone and AFM1, the sum of FUMs (FB1, FB), OTA, patulin, DON and ZEA [11]. The MLs for feedstuffs were set as mentioned above mycotoxins, except for patulin and FUMs [12].

Maize is one of the major feedingstuffs in the world because of its importance as a main source of energy and protein in animal feeding. In relation to the previous year in Serbia crop production decreased by 23.5 % however, maize is one of the most important agricultural products, both by its production and by the profit it generates in foreign trade. During the 2015–2017 season was 5,4 t/ha, 7,3 t/ha and 4,0 t/ha, respectively [4]. Damage in the production and export of animal feed and dairy products from Serbia caused by contamination of milk and corn by aflatoxins during 2012-2013. are estimated at around 100 and 125 million euros. Costs arising from increased sampling and analysis, as well as investing in additional research can not be estimated. On the basis of rough indicators, economic damage due to lack of adaptatione strategies of Serbian agriculture on the resulting climatic changes, it can be concluded how important is the development of preventive models for preventing food contamination of molds and mycotoxins [10]. Knowledge of environmental factors which support fungi to colonize, growth, and interact with plants is important in order to better understand the variation in the population structures of mycotoxigenic fungi and their ability to produce mycotoxins. Thus, climate change has the potential to increase the risks of new fungal genotypes, usualy involved in food and feed safety.

Occurrence, significance, and toxicity of mycotoxins

The most important agro-economic and public health classes of mycotoxins are aflatoxins (AFTs), ochratoxin A (OTA), zearalenone (ZEA), trichothecenes (TCT), and fumonisins (FUMs) produced by species of *Aspergillus*, *Penicillium* and *Fusarium* [13,14]. In this section we tries to briefly review the important classes of mycotoxins, incidence related to climate changes and discuss to their role in public health risk assessment. Considering that AFTs, DON, FUMs, ZEA, TCT, and OTA are the most frequently studied mycotoxins, on which there are more data, in Tables 1 to 5 an overview of mycotoxins occurrence in different commodities in Serbia since 2010 is presented.

Aflatoxins

Mycological examination of barley, maize, soybean, sunflower, and wheat from different environments in Serbia during 2008–2012 showed that themost frequently isolated genus was *Aspergillus*, particularly *A. flavus* and *A. niger*, although other *Aspergillus spp.* were very rarely detected. Depending on environmental conditions, the highest average frequency of *A. flavus* on the analyzed grain occurred in 2012, then in 2010, and was much lower, and with similar levels in 2008, 2009, and 2011. Before 2008, *Aspergillus spp.* in Serbian grain occurred mostly at low frequency and incidence, but the very high temperatures and extreme drought in 2012 caused *A. flavus* to occur in epidemic proportions [8]. Table 1 and Figure 3 summarizes studies on the incidence of aflatoxins in food groups and milk in Serbia.

Aflatoxins (AFTs) are difuranocoumarin derivatives primarily produced by *Aspergillus flavus* and *A. parasiticus fungi*, which contaminate agricultural commodities [15]. To date, nearly 18 different types of aflatoxins have been identified the five predominant ones being aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and AFM1 (based on their fluorescence under UV light, blue or green). AFM1 is transformed at the hepatic level by cytochrome P450 enzymes and excreted into the milk in the mammary glands of both humans and lactating animals after the animals have ingested feeds contaminated with AFB1. AFM1 is relatively stable during pasteurization,

sterilization, and storage of milk and milk-based products. Among these toxins, AFB1 is the most predominant and the most potent hepatocarcinogen and has been classified as a Group 1, known human carcinogen [16,17], while other compounds have lower toxicity, carcinogenic, mutogenic, and teratogenic effects. For this type of carcinogen, it is generally felt that there is no threshold dose below which no tumour formation would occur. In other words, only a zero level of exposure will result in no risk.

Recent results support hypothesis that some species might shift their geographical distribution in response to global warming, leading to changes in the pattern of mycotoxin occurrence. Developing crops are usualy resistant to infection by *Aspergillus spp* and related mycotoxins, unless environmental conditions favour fungal growth and crop susceptibility. Periods of drought combining with high temperatures significantly increases AFs production in the field. Recent report [18,19] predicted that, within the next 100 years, aflatoxin B1 will become a food safety issue in maize in Eastern Europe, Balkan Peninsula and the Mediterranean regions, especially under a +2 °C scenario. Since weather conditions in 2012 were favorable for *Aspergillus* mold growth, a larger percentage (68.5 %) of maize harbored AFs at levels 1μg/kg. According to Serbian and EU regulation only 46.5 % of maize could be used for human consumption, while maize (24 %) with AFs in the range of 10–50 μg/kg could be used only for animal feed, according to Serbian regulation. Due to the severity of the corn contamination, elevated concentrations of AFM1 were found in milk [20]. Elevated temperatures and extreme weather events, mentioned above, have directly and indirectly impact on the dairy industry. In such climate conditions as a consequence, the contamination risk for maize-derived products and for milk will be higher than in the past, particularly under inadequate storage conditions [21].

Table 1. The incidence of AFT in food and feed samples

Commodity	Region	N (%)	Range (μg/kg)	Mean (μg/kg)	Ref.
Maize	All regions		0.33-2.40		[22]
			1.01-86.1	363	[23]
			Up to 560	33,21	[20]
			2.31-3.34 (harvested)		[24]
			1.03-4.11 (stored)		
			1.98-7.01	1,33	[25]
	West Backa	57.2	1.3–88.8		[26]
	North Banat	13.9	0.60–2.8		
	South Banat	5.6	1.8–28.5		
	Central Serbia	2.8	2.1–7.5		
	Vojvodina	5	2.28-4.31	3.22	[27]
		5-72.3	1.0–111.2		[28]
Flours of various cereals	Serbian market	5.2	1.59–4.76	2,13	[29]
Maize flour		48.2	max. 9.14	0.55	

N-number of positive samles (%-percentage of positive samples)

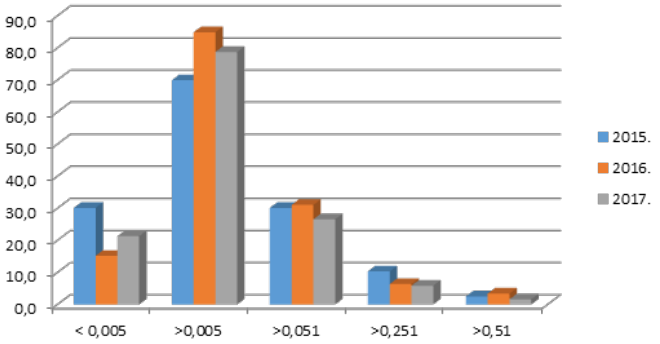

Figure 3. Incidence (%) of aflatoksin M1 contamination during three years period of investigation [21].

Fumonisins

A number of *Fusarium* species particularly F*usarium verticilliodies* and *F. proliferatum* produces fumonisins (FUMs), a group of some 28 compounds. These compounds are predominantly produced in maize and other cereal grains. Due the similarities in favorable fungal growth conditions (high temperatures in humid climates), fumonisins often co-occur with AFs especially in corn [30]. Although 28 FUM analogs have been identified, fumonisin B1 (FB1) is the most predominant and well-studied isoform which have a longchain hydrocarbon unit (similar to that of sphingosine and sphinganine) playing a role in their toxicity. FB1 exposure has been associated with liver and esophageal cancers in high-risk populations therefore has been classified as a Group 2B, possible human carcinogen [31]. FBs have also been implicated as a risk factor for neural tube defects (NTDs), while in animals FUM scause equine leuko encephalomalacia and porcine pulmonary edema. Last report indicates that Serbia is one of the few regions in Europe with proven cases of ELEM [32]. The mechanism of action of FB1 induced neurotoxicity is the inhibition of the enzyme ceramide synthase an enzyme responsible for the acylation of sphinganine and

Table 2. The incidence of FUMs in food and feed samples

Commodity	N (%)	Range (μg/kg)	Mean (μg/kg)	Ref.
Wheat		750–2465		[36]
Corn		30–1520	176	[37]
		880–2950		[38]
		520–5800	1730	[30]
		1519–9780		[24]
		760–35760		
Hors feeds		1680-6050	7,73	[32]
Corn	83	Up to 20340	1009	[39]
Pig feed	3 (100)	350-1061		[10]
Corn	74	540.1-5076	2750	[27]
Corn flours	96.4	15-1468.5	205.5	[40]
Corn flake	73.3	15-579.4	87.3	

N-number of positive samles (%-percentage of positive samples)

sphingosine [33]. The World Health Organization [34] established a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg of b.w. for fumonisins B1, B2 and B3, alone or in combination. Agri-climatic conditions (cool and wet summers) in Serbian farming, including among others conditions influence occurrence of *Fusarium spp.* and related mycotoxins. Incidence and distribution of FUMs concentration in examined samples during period of investigation are presented in Table 2. The overall prevalence of FUMs in Serbia, for the investigated period, was slightly higher compared to the prevalence of FUMs in samples collected between 2000 and 2010 [35].

Zearalenone

Zearalenone (ZEA) is a phenolic resorcyclic acid lactone with potent estrogenic properties, produced by several species of *Fusarium* mainly occurs in wet temperate weather associated with improper storage condition (high moisture) [41]. Among the cereals in which ZEN can occur, maize has been shown to have the highest contamination levels, particularly in central European countries such as Serbia (Table 3). Hence ZEA is one of the most common contaminants of feed and its components. It is also the most prevalent of the mycotoxicoses in domestic animals. These results can be explained by the heavy total rainfall during the maize harvest in 2014 and 2016, mild winter during 2015 as well as uncontrolled conditions of temperature and relative humidity during the storage, which caused the intensive development of mold and increased the content, particularly of *Fusarium* mycotoxins in stored maize [35]. ZEA and its major alcohol metabolites α-zearalenol and β-zearalenol share structural similarity with the human sex hormone 17b-estradiol. Therefore, exposure to this mycotoxin has been linked to estrogenic activity in human and animals likely due to the estrogenic activity exerted by ZEN and its metabolites upon interaction with the hepatic, uterine, mammary, and hypothalamic estrogen receptors. Pigs and cows are very sensitive to ZEA whereas poultry are very tolerant. Although IARC found limited evidence of ZEA carcinogenicity in animal models, classifying it mycotoxin as non carcinogen to humans-Group 3 [42], the SCF [43] established a tolerable daily intake (TDI) of 0.25 μg/kg b.w based on recent data in the most sensitive animal species.

Table 3. The incidence of ZEA in food and feed samples

Commodity	N (%)	Range (μg/kg)	Mean (μg/kg)	Ref.
Wheat		10–1000		[36]
Corn		1.79–3.39		[37]
Wheat flour		1.9–21.1	4.6	[44]
Corn		15.44–188.05		[38]
	15	35.6-183.5	83	[27]
Corn flours	66,1	1-242.1	15	[40]
Corn flake	86,7	max. 121.6	13.6	

N-number of positive samles (%-percentage of positive samples)

Trichothecenes

Trichothecenes are toxic metabolites majorly produced by *Fusarium spp.* The TCT mycotoxins comprise a vast group of more than 100 fungal metabolites classified based on the substitution pattern of the tricyclic 12,13-epoxytrichothec-9-ene (EPT) in four groups, A, B, C and D. Although the group of TCTs has been thoroughly studied worldwide, in Serbia, more intensive studies on DON were initiated after 2005 [45]. In a study recently carried out in Serbia (Table 4), DON was affecting several major cereal crops including oats, barley, corn, and wheat. The overall prevalence of TCT toxins in Serbian cereals for the investigated period was slightly higher compared to the prevalence of these mycotoxins in samples collected between 2005 and 2010. Weather conditions recorded in 2010 and 2014, in terms of air temperature and the amount of precipitation, had a significant influence on TCT occurrence [46]. The EPT structure is considered essential for toxicity. At the cellular level TCT cause protein synthesis inhibition by affecting the 60S subunit of the ribosome interfering with the peptidyl transferase activity [47]. Despite IARC in 1993 designated DONas a Group 3 (not classifiable) human carcinogen due to inadequate evidence of animal carcinogenicity, and lack of investigation in humans, TCT are known to cause neurotoxicity, immunosuppression and renal toxicity. The Joint Food and Agriculture Organization/ World Health Organization (FAO/WHO) Expert Committee on Food Additives established PMTDI for DON and its acetylated derivatives (3-ADON and 15ADON) of 1 μg/kg-1 b.w., whereas for NIV and the sum of T-2 and HT-2 toxins the SCF proposed a temporary tolerable daily intake TDI of 0.7 and 0.06 $\mu g/kg^{-1}$ b.w., respectively [48]. Recently, the SCF [50] concluded that a full TDI of 0.1 $\mu g/kg^{-1}$ b.w. for the sum of T-2 and HT-2 toxins can be now established, based on recent data.

Table 4. The incidence of TCT in food and feed samples

Commodity	N (%)	Range (μg/kg)	Mean (μg/kg)	Ref.
Wheat	14 (82.4)	68–1572		[50]
		50–5000		[36]
		25–135.6		
Wheat flour		17.5–976	325	[44]
		9.8–26.9	4.1	
Crop maize	52.0	25.3–200	154.1	[51]
Corn		41–226		[38]
		600–700	650	[30]
		25.09–209	50.93	
		380–10.684		[33]
		42–238		
Crop wheat	100	175.0–1440.0	762.5	[52]
Corn	52	275.2-882.1	541	[27]
Crop maize	96.0	264.4–9050.0	3063.3	[53]
	15.5	252.3–6280.0	921.1	
Corn flour	42,9	25-931.8	101.3	[40]
Corn flake	40	25-878.6	255.1	

N-number of positive samles (%-percentage of positive samples)

Ochratoxin

The ochratoxins are a group of related pentaketide metabolites comprises a dihydrocoumarin moiety linked to a molecule of L-β-phenylalanine via an amide bond, mainly produced fungi of the genera *Aspergillus* and *Penicillium* [13]. Ochratoxins A have been isolated from a wide range of commodities all over the world, in both warm and cool climates, and are common contaminants of staple food crops, and beverages such as beer and wine and foods from animal origin, particularly pork or dry-cured meat products [14,54]. OTA is a potent nephrotoxin and based on animal evidence has been causative agent of porcine nephropathy [55]. Historically, OTA has been implicated in several nephropathies, most noticeably Balkan Endemic Nephropathy in the former Yugoslavia, chronic disease, and endemic nephropathy associated with urothelial-cancer. OTA is classified as a possible human carcinogen (group 2B) by IARC (1993) on the basis of sufficient evidence of carcinogenicity in animal models, but insufficient evidence from human studies. OTA was last evaluated by the Scientific Committee on Food (SCF) in 1998 when it concluded that OTA possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic, and possibly neurotoxic properties. The mechanism of action seems to be related to the formation of DNA adducts [56]. Based on this assessment, a tolerable weekly intake of 120 ng/kg b.w. was derived for OTA. Due to the high health risks of OTAs, they have been studied more often than other mycotoxins in our region. Results found in study by Milicevic et al., [57] suggest that in general, OTA contamination in pork and chicken meat originating from different part of Serbia is low and hence for the consumer the contribution to the total intake of OTA from pig and chicken products is very small compared with other sources. The values are far below the Acceptable Daily Intake (ADI) of these toxins (Table 5).

Table 5. The incidence of OTA in food and feed samples

Commodity		N (%)	Range (µg/kg)	Mean (µg/kg)	Ref.
Blood plasma (pigs)		38 (30)	0.24-228	3.70	[57]
Liver	pigs	24 (26,6)	0.18-14.4	0.64	
	chicken	23 (38.33)	0.14-3.90	0.41	
Kidney	pigs	40 (33.3)	0.17-52.5	1.24	
	chicken	17 (28.33)	0.10-7.02	0.36	
Chicken gizzard		16 (26.6)	0.25-9.94	0.36	
Chickens feed		100	19.04–51.30	34.40	[58]
Hens feed		100	28.34–65.30	43.89	
Breakfast cereals		20.7	0.07–11.81	1.76	[30]

N-number of positive samles (%-percentage of positive samples)

Other important mycotoxins with fewer occurrences

Other important mycotoxins that can be found as contaminants of foods include patulin, citreoviridin, gliotoxin, griseofulvin, mycophenolic acid, b-nitropropionic acid, Kojic acid, penitrems, penicillic acid, viomellein, vioxantin and xanthomegnin and walleminols. Also, emerging mycotoxins such as fusaproliferin, beauvericin, enniatins and moniliformincan be contaminants of foods. In Serbia, there are not current study on the presence of these mycotoxins in foods, likely due to the lack of research to determine their occurrences as well as potential human and animal health effects.

Current and future outlook

Although the emphasis over the past decade has been on microbial food safety issues, in this new global environment chemical hazars has made the headlines over the past several years. The impact of mycotoxins on public health program can be assessed by multiple criteria, such as the health and veterinary care costs, economical losses of livestock production, forage crops and feeds, regulatory costs, and research costs. Despite that developing countries are usually susceptible to mycotoxin outbreaks or interventions, developed nations are also at risk of exposure due to contaminated food imports. In a global climate changing we must also consider that fungal growth and consequently contamination of commodities by mycotoxins in uncommon places is likely to be occur. Temperature and rainfall are the climatic factors that are most likely to affect Serbia in future. These can be expected to have a wide range of impacts on plants and plant pathogens and to affect mycotoxin contamination in various commodities. Furthermore, the identification of climate factors on mycotoxins occurrence is central to risk management. From the Serbian perspective, further research should be focused on:

- the development of predictive models for mycotoxin occurrence based on regional weather data in order to estimate the risk of contamination after agiven growing season,
- continuous monitoring of mycotoxins level in animal feed, particularly in regions where milk samples were previously contaminated by AFM1 above the legal limit,
- improving analytical facilities, and implementing strict regulations, would avoid or reduce these natural contaminants in food and feeds and ensure the safety of food chain.

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AUTHOR INFORMATION

Dragan Milicevic – DVM, MSc, PhD, Senior Research Associate, Adviser of Director for scientific research. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia. Tel.: +381112650655. E-mail:dragan.milicevic@inmes.rs. *corresponding author
Brankica Lakicevic – MSc, PhD, Senior Research Associate, Department of microbiological and molecular - biological research. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia.Tel.: +381112650655. E-mail: brankica.lakicevic@inmes.rs
Radivoj Petronijevic – MSc, PhD, Research associate, Department of chemical and physical-chemical analyses. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia. Tel.: +381112650655. E-mail: radivoj.petronijevic@inmes.rs
Zoran Petrovic – MSc, Phd, Research associate, Coordinator of the Institute's Management System. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia. Tel.: +381112650655. E-mail: zoran.petrovic@inmes.rs
Jelena Jovanovic – DVM, Research assistant, Department of sensory and physical testing and parasitology. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia.Tel.: +381112650655. E-mail: jelena.jovanovic@inmes.rs
Srdjan Stefanovic – PhD, Research associate, Department for residues testing. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia.Tel.: +381112650655. E-mail:srdjan.stefanovic@inmes.rs
Sasa Jankovic – PhD, Research associate, Department for residues testing. Institute of meat hygiene and technology, 11040 Belgrade, Kacanskog 13 Serbia. Tel.: +381112650655. E-mail:sasa.jankovic@inmes.rs

Contribution

Authors equally contributed to the writing of the manuscript and are equally responsible for plagiarism

Conflict of interest

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Original scientific paper

STUDY OF GROUND PORK THAWING DYNAMICS USING MAGNETIC RESONANCE IMAGING

Vladimir M. Stefanovskiy[1]*, Evgeny V. Morozov[2], Vyacheslav M. Bouznik[3]

[1]All-Russian Scientific Research Institute of Refrigeration Industry – Branch of V.M. Gorbatov Federal Research Center for Food Systems of RAS, Moscow, Russia

[2]Institute of Chemistry and Chemical Technology of the Siberian Branch of the Russian Academy of Science – Branch of Federal Research Center "KSC SB RAS", Krasnoyarsk, Russia

[3]All-Russian Scientific Research Institute of Aviation Materials of the state research center of the Russian Federation, Moscow, Russia

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Abstract

Visualization of changes in shape and size of the frozen residue during the thawing of ground pork is implemented using magnetic resonance imaging. A technique has been developed to study the displacement of thawing front line without damage to integrity of the object being thawed. It has been established that the melting of bound water crystals outran the melting of free water crystals in meat. A mathematical model that adequately describes the experimental data obtained in the analysis of tomograms is proposed. Tomograms are an important information source for studying the patterns of heat and mass transfer during the thawing of frozen foods.

Introduction

Monitoring of continuous refrigeration chains indicates that transportation of frozen goods with numerous handling operations or emergencies causes partial (and sometimes complete) thawing of frozen products. Subsequent refreezing changes the structure-forming and water-holding properties, increases losses during thawing and reduces sensory quality of the thawed products. For effective control of these changes, it is necessary to conduct a system analysis of food thawing dynamics.

Traditionally, the rate of melting is defined as the ratio of thawing front line distance to the time. Monitoring of the phase boundary displacement may be achieved by using modern methods of internal imaging, such as optical coherence tomography, ultrasound imaging, X-ray computer tomography, magnetic resonance imaging (MRI), thermal imaging methods providing real-time information about the processes in the system [1]. Among these, MRI method is one of the most common and effective. MRI has proven itself both in medical diagnostics due to its non-invasiveness, safety and high informativity [2] and in food system studies including the impact of freezing and thawing on the quality of meat, fish and vegetable products [3,4,5,6,7,8].

The purpose of the work is to test the method of MRI imaging to assess the degree of thawing of minced meat, to identify the characteristics of the course of this process and to offer a method for calculating the thickness of the thawed layer.

Materials and methods

"Ideal product" model was used as a test subject for MRI studies, which was originally proposed in literature [9] in the form of a plastic cylindrical container filled with glass balls (d = 1 mm) and water. Container volume is 1.8 ml. The weight of the model sample contents was 4.37 g (the mass fraction of water was 22.9 %). A small amount of a paramagnetic substance containing gadolinium ions (paramagnetic contrast agent gadodiamide) was added to the water, the concentration of which did not exceed 20 ppm. This made it possible to significantly reduce the time of nuclear magnetic relaxation of water and increase the efficiency of the applied MRI techniques through the use of much shorter pulse sequences.

In the second stage of the study, the container was filled with ground pork. Ground meat weight was 1.67 g.

The samples were placed in a tomographic scanner sensor, frozen with nitrogen gas at a temperature of minus 30 °C and a speed of 0.15 to 0.25 m/s. Thawing was carried out with an air at room temperature (20 °C) at the same speed range. After a specified time, the current tomographic image was recorded.

Tomographic measurements were carried out in the Krasnoyarsk regional center for collective use using Bruker AVANCE DPX 200 NMR micro-tomographic scanner in the following configuration: shielded vertical superconducting magnet with an aperture of 89 mm and a magnetic field of 4.7 T; PH Micro 2.5 tomographic sensor with GREATE 3/40 amplifiers; water-cooled gradient

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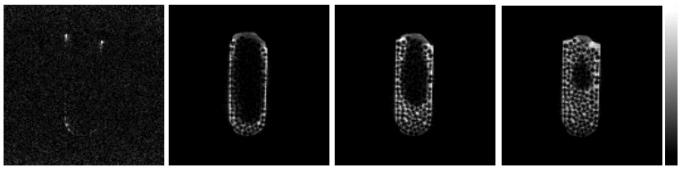
system with a maximum gradient of 1 T/m; a bird cage type radio-frequency coil with a diameter of 25 mm set to a proton resonance frequency of 200 MHz; Paravision 4.0 software. All tomograms were recorded by 1H nucleus. To obtain tomograms, we used spin echo based technique (Multi Slice Multi Echo [10]) with TR/TE parameters of 100/3 ms, field of view of 40 mm, matrix of 128x128 pixels, slice thickness of 1 mm, and time to obtain an individual image of 25 s.

Presults and discussion

MRI during the thawing of test subject showed that at the initial stage of sample heating, ice areas have almost no signal and are represented in tomograms by black color (Figure 1, image A, images B,C,D - demonstrate an increase in the thickness of the thawed layer in the form of a light area). Water droplet forming during the thawing process leads to the visualization of bright areas at the sample perimeter. As melting proceeds, the frozen part area gradually decreases until the ice melts completely.



A B C D

Figure 1. A series of model sample MRI images during thawing

The phase boundary may be visualized more clearly by a series of differential images when subtracting successive tomograms. Only the line of signal change will remain on the images, i.e. the desired phase transition front line (Figure 2).

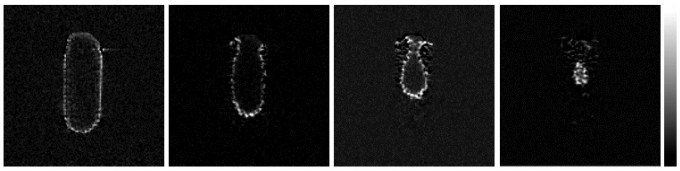


Figure 2. A series of model sample differential images during thawing

Tomograms reveal the characteristics of the shape change of the sample frozen part, the geometry of which differs from the original geometry of the frozen sample (cylinder). This phenomenon will be the subject of further research.

The developed visualization method allows not only to study the dynamics of ice melting in a model sample, but also to identify the characteristics of meat and meat products thawing. Also using mathematical methods of image processing, it is possible to quantify the volume of the frozen part and its dynamics during thawing.

Our observations of the ground meat thawing showed that at the initial stage, the signal intensity gradually increases almost in all parts of the sample. This may be explained by the fact that in addition to water molecules, a large number of proton-containing molecules (proteins, fats, carbohydrates) are present in meat, which contribute to overall signal intensity in the image, even for a frozen object. While water molecules in ice are considered to be non-labile, biological molecules retain some lability due to the segmentation movement of biopolymer chains.

It is well known that water in biological tissues is represented in various states including free and bound ones with varying degrees of binding. [11]. Thus, water molecules in a gel-like state (water in collagen macrostructure and other tissues) also retain sufficient lability at negative temperatures, unlike water molecules in the structure of ice. This leads to the conclusion that during thawing of ground meat, a gradual increase in the signal intensity is observed throughout the sample due to increase in the local dynamics of bound water molecules and improvement in the dynamics of the segmentation movement of biopolymer chains.

Consequently, when the conditions for melting of ice microcrystals (free water between the meat structural elements) are achieved, the target front line may be visualized only by differential images (Figure 3, the differential images are presented in color for greater clarity).

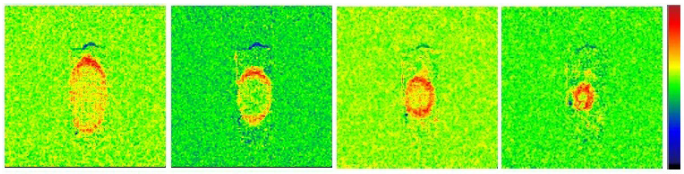


Figure 3.A series of the ground meat sample differential images during thawing

The analysis of tomograms shows that the frozen part of the sample (of a small size) is quickly heated to a cryoscopic temperature, while the moving interface between the frozen and unfrozen areas remains until the end of melting.

Another important aspect of the problem under study is a quantitative assessment of the thawing dynamics. The solution of this problem is associated with the construction and analysis of a mathematical model for the process. Frozen object thawing is mathematically formulated as follows [12]:

$$\frac{\partial t_1(x,\tau)}{\partial \tau} = a_1 \frac{\partial^2 t_1(x,\tau)}{\partial x^2} \quad (\tau > 0;\ 0 < x < \xi), \tag{1}$$

$$\frac{\partial t_2(x,\tau)}{\partial \tau} = a_2 \frac{\partial^2 t_2(x,\tau)}{\partial x^2} \quad (\tau > 0;\ \xi < x < \infty), \tag{2}$$

$$t_2(x,0) = f(x), \tag{3}$$

$$t_1(0,\tau) = \varphi(\tau), \tag{4}$$

$$t_1(\xi,\tau) = t_2(\xi,\tau) = t_3 = const., \tag{5}$$

$$\frac{\partial t_2(\infty,\tau)}{\partial x} = 0 \tag{6}$$

At the phase boundary

$$-\lambda_1 \frac{\partial t_1(\xi,\tau)}{\partial x} + \lambda_2 \frac{\partial t_2(\xi,\tau)}{\partial x} = r\rho_2 \frac{\partial \xi}{\partial \tau} \quad (7)$$

where index of 1 refers to the thawed layer with a thickness of ξ; index of 2 refers to the frozen part of the object; λ is the coefficient of thermal conductivity, W/(m K); r is the specific heat of thawing, J/kg; ρ is the density, kg/m3; t t3 is the cryoscopic temperature, °C; τ is the time, s.

The problem of heat transfer by heat conduction as an example of conjugation of two temperature fields of the frozen and thawed object areas upon special conditions on the moving phase boundary was solved for the first time in 1831 by the members of the Russian Academy of Sciences, Prof. Lame and Prof. Clapeyron, and later in 1889 by the mathematician from Vienna, Stefan [12]. The differential equation expressing the relationship between the thickness of the thawed layer ξ and the thawing time τ is as follows:

$$r\rho_2 \frac{d\xi}{d\tau} = \frac{\lambda_1(t_c - t_3)}{b + \sqrt{\pi a_1 \tau}\ erf \frac{\xi}{2\sqrt{a_1\tau}}} \exp\left(-\frac{\xi^2}{4a_1\tau}\right) - \frac{\lambda_2(t_3 - t_4)}{\sqrt{\pi a_2 \tau}\ erfc \frac{\xi}{2\sqrt{a_2\tau}}} \exp\left(-\frac{\xi^2}{4a_2\tau}\right) \quad (8)$$

where $b = \lambda_1(1/\alpha + \delta_{ст}/\lambda_{ст})$ is the coefficient of thermal resistance at the boundary of the thawed layer and the container wall; α is the coefficient of heat transfer from air to the container; δст and λст are the thickness and the thermal conductivity of the container wall.

For ground meat at cryoscopic temperature and heat transfer in the boundary conditions of the third kind, the equation (8) has the following form:

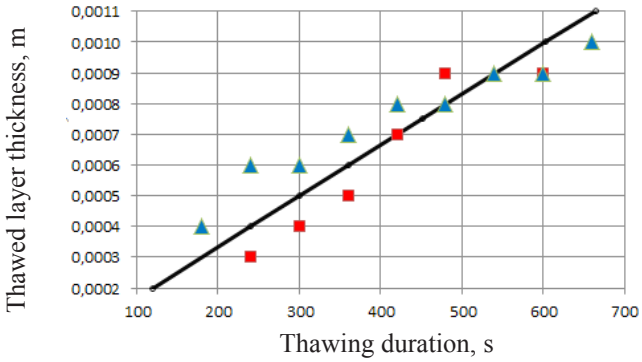
$$r\rho_2 \frac{d\xi}{d\tau} = \frac{\lambda_1(t_c - t_3)}{b + \sqrt{\pi a_1 \tau}\ erf \frac{\xi}{2\sqrt{a_1\tau}}} \exp\left(-\frac{\xi^2}{4a_1\tau}\right) \quad (8)$$

By decomposing exp and erf functions into series in (9) and limiting them to the first members, the following relation is obtained:

$$\tau = \frac{r\rho_2}{\lambda_1(t_c - t_3)}\left(\frac{\xi^2}{2} + b\xi\right) \quad (9)$$

Due to certain assumptions when obtaining a private relationship (10), it is appropriate to verify the adequacy of this mathematical model. When performing calculations, the thermophysical characteristics of meat are taken from [13,14,15,16]: r = 130,000 J/kg; ρ2 = 964 kg/m3; λ1 = 0.45 W/ (m K); defrosting environment tc = 20 °C; t3 = -1 °C; α = 10 W/(m2 °K); δст = 0.0001 m; λст = 0.2 W/(m K).

Figure 4 presents the comparison of experimental data with a theoretical solution (10).



Comparison of the results obtained by analytical and experimental methods indicates the possibility of a dialogue with computer, i.e. to analyze alternatives, verify hypotheses, carry out experiments with mathematical models significantly increasing the efficiency of engineering development in creating fundamentally new refrigeration technologies and systems.

Conclusions

The visualization of the thawing boundary displacement in a frozen object indicates that magnetic resonance imaging may be used in the industry to determine the degree of meat product thawing in the case of violation of the refrigeration chain continuity.

Comparison of the results obtained by calculation and experimental method proves the validity of the theoretical equations and the possibility of their use in the computer simulation of thawing processes.

A series of tomograms over time is not only a source of information, but also a tool for monitoring the process of industrial thawing of frozen products.

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AUTHOR INFORMATION

Vladimir M. Stefanovskiy - doctor of technical sciences, professor, head scientific collaborator, All-Russian Scientific Research Institute of Refrigeration Industry – Branch of V.M. Gorbatov Federal Research Center for Food Systems of RAS. 125422, Moscow, Kostyakova str., 12. Tel.: +7-495-253-15-74. E-mail: stef-vm@yandex.ru *corresponding author
Evgeny V. Morozov - candidate of physical and mathematical cciences , senior researcher, Institute of Chemistry and Chemical Technology of the Siberian Branch of the Russian Academy of Sciences - Branch of Federal Research Center "KSC SB RAS". 660036, Krasnoyarsk, Akademgorodok 50/24. Tel.: +7-391-249-85-61. E-mail: morozov_if@mail.ru
Vyacheslav M. Bouznik – doctor of chemical sciences, professor, academician of RAS, advisor to the general director, All-Russian Scientific Research Institute of Aviation Materials of the state research center of the Russian Federation. 105005, Moscow, Radio str., 17. Тел.: +7-977-969-70-30. E-mail: bouznik@ngs.ru

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Review paper

LOW-TEMPERATURE ATMOSPHERIC-PRESSURE PLASMA IN MICROBIAL DECONTAMINATION AND MEAT TECHNOLOGY. A REVIEW

Natalia A. Gorbunova

V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia

Keywords: *low-temperature plasma, plasma-based inactivation, microorganisms, meat products, nitrite*

Abstract

The development of plasma technology is associated with the unique features of non-equilibrium low-temperature plasma: high electron energy and high concentration of chemically active excited and charged particles at low gas temperature, which allows to process thermolabile materials and biological objects in gentle conditions when high temperatures are not required. The biological effects of low-temperature plasma exposure are considered. It was established that during plasma treatment, a combined effect on cells and tissues of living systems from UV radiation, ions and chemically active particles occurs. Depending on the plasma type, the significance of each of the listed mechanisms for increasing the effectiveness of plasma treatment may vary. However, all these mechanisms interact with each other and have a synergistic effect. It was shown that the conducted studies confirm the ability of low-temperature plasma to inactivate pathogenic microorganisms upon contact with biological objects and foods. The results of the studies are presented, the purpose of which was to study the effect of plasma treatment on nitrite concentration in the water treated by this method and to assess the possibility of its use as a source of nitrite when curing meat products.

Introduction

In recent years, cold plasma technology has been used in chemical technology, metal processing and production of various coatings, medicine, and food industry, in particular, as a low-temperature sterilization method that provides a high level of microbial inactivation for water purification and disinfection of premises. The potential size of the cold plasma equipment market in Russia and the EU amounts to $22 billion with a growth potential of 7 to 8 % per year [1]. To implement these technologies in industry, various plasma installations have been developed, in which thermal and plasma-chemical treatment of substances is realized.

The technology for cold plasma production with the temperature of the torch visible part of 40 to 42 °C is known for a long time. The main difference between cold plasma and other plasms types is that the temperature of the former is close to the temperature of biological objects. This fact allows applying this type of plasma to living systems [2].

1. The concept of plasma

Plasma is often considered the fourth physical state of matter. The English chemist and physicist W. Crookes introduced this concept in 1879 to describe the ionized medium of a gas discharge. When solid substance is heated, it passes into a new state, usually liquid. In turn, the liquid passes into a gas when heated. Its further heating leads to the ionization of atoms. Gas in which a significant part of atoms or molecules is ionized is called plasma [3].

Plasma is a complex, quasi-neutral multicomponent system including plasma radiation, both positively and negatively charged particles, and electroneutral particles, which, however, are chemically active (radicals, excited atoms and molecules). Thus, plasma acts simultaneously as a source of radiation, part of which has bactericidal properties, and as a chemically active medium [4, 5].

Electrolyte solutions, semiconductors and electric-discharge plasma generated in the range of low and atmospheric pressure of 10-2 to 103 Pa have similar properties. At this pressure, the plasma is a partially ionized gas with a degree of ionization less than 10-4, in which the concentration of electrons, positive and negative ions, radicals is sufficient to maintain a quasi-neutral state [6].

Plasma is often classified into low-temperature and high-temperature, as well as into equilibrium and non-equilibrium. Low-temperature plasma, in turn, is divided into thermal and non-thermal plasma. In non-equilibrium plasma, electron temperature significantly exceeds the total temperature of ions. In equilibrium plasma, the total values of both temperatures are approximately equal, while the equilibrium plasma is often hot [7].

Particles produced in high-temperature plasma are in a state of thermal equilibrium, and, as a result, electrons and heavy ions have a high temperature. Low-temperature thermal plasma is characterized by the equality of electron and gas temperature, which is based on elastic collisions between electrons and heavy particles (ions, molecules,

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and atoms). The energy of the electrons is consumed by heavy particles, which leads to their heating. In a thermal plasma, electrons and heavy particles are in the state of local thermodynamic equilibrium.

2. Some aspects of the low-temperature plasma physics

In the plasma state of a gas, vibrational-rotational excitation and dissociation of the molecules occur. Particles formed as a result of electron impact are highly reactive and interact not only with each other, but also with any object introduced into plasma. The plasma state of a substance, even with a low degree of ionization, gives a wide range of chemically active particles, the source of which is not thermal energy used in classical technological processes, but the energy of an external electric field. This causes the so-called non-equilibrium of the electric-discharge plasma due to the low efficiency of energy transfer from the electron to other particles by collisions. As a result, a difference occurs between the translational energy of electrons and more massive particles (ions, radicals). Recalculation of the average electron energy into the corresponding thermal units gives the electron "temperature" of about 3·104 to 1·105 K [8].

Low-temperature plasma is considered equilibrium if its components are in thermodynamic equilibrium, i.e. the temperatures of electrons, ions and neutral particles are equal. In a low-temperature plasma, non-equilibrium conditions are easily created as a result of the selective action of external electric fields: the electrical energy from them is transferred to charged particles, and then to gas particles by collisions. With this method of energy introducing, the average energy of charged particles may significantly differ from the thermal energy of neutral particles. First, this refers to electrons, which, due to their small mass, inefficiently exchange energy during elastic collisions with neutral gas particles. In this case, not only the average electron energy, but also the form of energy distribution of electrons may significantly differ from the equilibrium [7].

For non-thermal plasma, it is characteristic that the temperature of electrons is significantly higher compared to the temperature of ions and neutral gas. This temperature difference depends on the frequency of collisions between electrons and heavy particles. Due to the small loss of kinetic energy in elastic collisions between electrons and heavy particles, the electron energy remains high. Therefore, non-thermal plasma is not in a state of local thermodynamic equilibrium. Because the temperature of neutral gas is equal to or close to room temperature, non-thermal plasma is often called a "cold" plasma [9].

In a non-thermal plasma, free electrons are very "hot" and have temperatures of several thousand degrees Kelvin (K), while neutral particles and ions remain "cold". Due to high-energy collisions, excited electrons form a whole spectrum of radicals and excited particles with high reactivity. This combination of low temperatures with high reactivity makes non-thermal plasma a technologically advantageous and very effective tool for performing technological processes that would require the use of very high temperatures and harmful corrosive chemicals even if they were feasible without plasma [9].

An important feature of non-equilibrium cold plasma is its ability to generate a unique 'one pot' cocktail of biologically active agents, such as reactive oxygen species and reactive nitrogen species, while remaining close to ambient temperature, which enables its safe application to biological materials including foods. Reactive particles and their concentration in plasma will vary depending on many factors including the gas, in which the plasma is induced, the configuration of plasma source, the consumed power of gas, the duration of treatment and the moisture level in the product [10].

Depending on the mechanism for obtaining low-temperature plasma, the following types of discharges are distinguished: a glow discharge, a dielectric barrier discharge, a corona discharge, and plasma jets. Non-toxic gases such as argon, helium, nitrogen, oxygen, air, water vapor, and their combinations are most commonly used as gas mixtures.

3. Biological effects of exposure to non-equilibrium low-temperature plasma

It has been established that during plasma treatment, a combined effect on cells and tissues of UV radiation, ions and chemically active particles is observed [11,12]. Depending on the type of plasma, the significance of each of these mechanisms for sterilization efficiency may vary. However, all these mechanisms interact with each other and have a synergistic effect [13,14].

Ions and chemically active particles interact with the outer layers of spores and bacteria, causing its erosion and loss of integrity. At the same time, chemically active particles and ions of the plasma-forming gas are adsorbed on the surface of bacteria and chemically react with the cell membrane molecules forming toxic compounds and secondary radicals. Such damage leads to the release of individual microorganisms from the matrix (dirt, biofilm, bacterial clump) on the surface of sterilized object. By removing the outer layers, plasma-induced erosion reduces shielding from UV radiation, which can cause direct destruction of the microbial genetic material.

Pulsed electrical discharges cause the formation of defects in the cell membrane of bacteria, which allows highly reactive oxidizing agents to freely penetrate into the internal environment of cells and disrupt their metabolic processes. This principle is used to inactivate microorganisms. Cells die as a result of ruptures in cell membrane that protects bacteria from the external environment [15,16].

The most significant biological effect of plasma on bacterial cells and living tissues is caused by the reactive

oxygen species (ozone, atomic oxygen, superoxide anion radical, peroxides, hydroxyl radicals) and reactive nitrogen species (for example, NO). In atmospheric-pressure plasma, reactive neutral particles (atomic oxygen, singlet oxygen, ozone) are mainly generated, and in low-pressure plasma, ions are predominantly produced.

The interaction of plasma electrons with cell membrane violates its structure. The accumulation of charged particles on the outer bacterial membrane may exceed its tensile strength and, thus, cause its damage. This mechanism is most likely in plasma treatment of gram-negative bacteria; their membrane is thinner than that of gram-positive microorganisms, and its structure is not so well arranged [17,18].

According to some authors, UV radiation is the dominant mechanism of the bactericidal action of low-pressure plasma [15,19]. UV radiation with wavelengths less than 300 nm (220-280 nm) penetrates deep into cells and causes breaks in DNA leading to the death of microorganisms and inhibition of their reproduction [11]. In addition to ruptures, UV radiation contributes to the formation of thymine dimers in DNA molecules, which prevents the bacterial cell from replicating the genetic material [19,20].

A study by the Russian scientists [21] showed that the treatment of E. coli cells with cold plasma results in partial or complete loss of integrity of the cell cytoplasmic membrane, which is accompanied by the release of intracellular compounds into the extracellular environment. Quantitative assessment of the cell membrane damage showed that a loss of at least 23.6 % of intracellular compounds is enough for cell death (calculated from the release of intracellular nucleotides). The use of media with different ionic strength to create osmotic shock showed that the treatment of E. coli cells with cold plasma resulted in a significant decrease of their membrane strength. The authors assumed that the cell membrane and cytoplasmic membrane are the first to be exposed to plasma active particles. Only after the active particles have passed through the damaged cell membranes, they may damage the DNA and key cell enzymes. At the same time, it was noted that the study of the mechanism for microbial inactivation by the cold plasma is only at initial stage. A clear understanding of this phenomenon will increase the efficiency of plasma-based sterilization, accelerate the widespread introduction of a new sterilization method and can help in solving current problems in the field of biosafety and in other areas where fast and effective microbial decontamination is required.

4. Use of low-temperature plasma as an antimicrobial agent. Plasma-based sterilization.

An important task is to determine the mechanisms and assess the effect of low-temperature plasma on microorganisms.

The advantages of plasma sterilization methods include the possibility of decontaminating materials that are sensitive to heat (the treatment temperature does not exceed 50 °C), the absence of auxiliary chemical compounds formation that are hazardous for the environment and human health [22,23] and a reduction in treatment duration [14].

It should be noted that at the current stage of research, the assessment of plasma-based microbial inactivation results obtained under various experimental conditions is a big problem [9].

For the first time, W. Siemens et al. [24] applied corona discharge for water purification from biological contaminations in 1857. Later, corona discharge efficiency was confirmed both in aqueous suspensions of bacteria [25] and in the sterilization of solid surfaces contaminated by microorganisms and their spores [26]. It has been established that for complete elimination of various gram-positive and gram-negative bacteria in corona discharge, 5 to 15 min exposure is necessary depending on the discharge power [8,13,14,21,27]. At the same time, the efficiency of plasma-based sterilization may be 99.9 %.

The efficiency and rate of microbial inactivation depends on the material, on which they are located. It was revealed that the most quickly inactivation of Escherichia coli K12 occurred on a polypropylene surface followed by glass and agar, respectively. An increase in the sensitivity of microorganisms with a decrease in pH and ambient temperature was noted [28]. Moreover, gram-negative microorganisms are more susceptible to plasma than gram-positive ones, which may be due to the different structure of the cell membrane [29].

Glow discharge has a bactericidal effect not only on single bacteria, but also on biofilms, which are extremely resistant to traditional sterilization methods. Thus, glow discharge destroyed the biofilms of *Rhizobium gallicum* and *Chromobacterium violaceum*, which were formed for four and seven days, causing the death of 100 % microorganisms [30,31]. It is especially important that glow discharge may be used when the treated surface is sensitive to external influences (for example, when treating food products), which prevents changes in color and structure and the formation of toxic products [31].

In Tomsk State University [5], the effect of atmospheric-pressure plasma treatment of surfaces contaminated with E. coli was investigated. A high-frequency discharge in air at atmospheric pressure was used as a plasma source. The generator created high-voltage pulses with a frequency of 122 kHz. The plasma was ignited 0.7 and 2.7 cm from the substrate, on which a layer of bacteria with a known concentration was applied. After irradiation, the substrate was placed in thermostat for 2 days. On the third day, microorganism colonies were counted to determine the effectiveness of sterilization.

The results of the experiments allow to conclude that the key factors of atmospheric-pressure plasma sterilizing effect under the above conditions on bacterial cultures

are ultraviolet radiation with 200 <λ <220 nm and electroneutral chemically active particles. Effectiveness of this UV radiation band is due to the fact that the maximum inactivation of microbial DNA occurs in this frequency range. The author notes that the latter is particularly interesting, since the plasma radiation intensity in the band of 200 <λ <220 nm is about 7 %, while the main bactericidal effect occurs at this frequency range.

For sterilization of products made from capillary-porous materials, high-frequency capacitive discharges (HFCD) of low pressure are most suitable, allowing for bulk treatment of materials due to volumetric non-independent pulsed-periodic microdischarges inside the pores [32].

The effectiveness of the bactericidal effect of HFCD plasma in the air depends on the initial concentration (N0) of the test culture and the duration of treatment (t). Therefore, the survival rate of *E. coli* at N0 ≈ 1 x 103 CFU/ ml and t = 5 to 10 min is 0.2 %, while for N0 ≈ 1.4 x 104 CFU/ml and t = 7 to 10 min is 71 %. The plasma effect on *S. aureus* is significantly lower: the survival rate of microorganisms with a 10-minute treatment is 83% for N0 = 1.8 x 104 CFU/ ml and 36 % for N0 = 8.0 x 103 CFU/ ml. The sterilizing effect of plasma exposure for *B. subtilis* strains with N0 = 1.7 x 102 CFU/ml is also low at t = 5 min: viable cells were isolated in an amount of ≈ 70 %. At t = 7 to 10 min, the survival of *B. subtilis* is about 2 to 3 %. For *C. albicans* with N0 = 1.0 x 103 CFU/ ml, a complete sterilizing effect was observed for all the durations of exposure to HFCD plasma.

R.V. Yakushin [8] studied the effect of barrier and spark discharges (amplitude 2.5 to 6 kV; frequency 45 kHz; flow rate 0.6 m3/h) on aqueous solution models containing *E. coli* with a concentration of 7 x 106 CFU/ml. It was shown that the barrier discharge under the given experimental conditions was ineffective against gram-negative bacteria. High inactivation of *E. coli* was noted after exposure of solution to a spark discharge. After the first two treatment cycles, a decrease in the concentration of bacteria by more than 95 % compared to initial level was demonstrated.

Treatment with spark discharge allows to almost completely inactivate microorganisms in water. The experiment showed the effectiveness of spark discharge for disinfecting water at extremely high concentrations of *E. coli* strain. There was a decrease in the concentration of microorganisms by seven orders over 10 treatment cycles.

It may be assumed that microbial inactivation is due to oxidative destruction and integrity violation of cell membranes under the influence of the chemically active oxidant particles formed. The oxidizing agents diffusing from the water-air interface into the treated solution penetrate into microorganism cells and react with the vital macromolecules involved in metabolic processes. It is suggested that this is accompanied mainly by the oxidation of amino acids and proteins, depolarization of nucleic acids, and decomposition of other biologically active substances [8].

One of the possible factors of microbial death under the action of spark and barrier discharges is damage to the cell and its structures due to thermal or mechanical effects. Shock waves are able to intensify chemical polymerization processes and breaking of chemical bonds in the cell, thereby destroying its membrane. Moreover, there is a rupture of the bacterial cell itself [8].

Since 1990, there has been a growing interest in the use of plasma jets to inactivate bacteria, since they are best suited for practical use because of their small size and easy plasma excitation. For microbial sterilization, the most actively used plasma jets are so-called "atmospheric-pressure plasma jets".

In preliminary experiments [33], the efficiency of plasma beam for inactivating bacteria was shown. In this study, *E. coli* bacteria were treated with plasma pencil in two different gaseous media (helium and helium mixed with 0.75 % oxygen) during different time (30 and 120 s). It was established that the area of the inactivated region increases with increase in exposure time, as well as with the addition of oxygen to helium, especially with increase in exposure time.

When assessing the antibacterial effects of plasma irradiation in the Scientific and Practical Center of Hygiene (Minsk, Republic of Belarus) [34], *Staphylococcus aureus* ATCC 6402 and *Pseudomonas aeruginosa* ATCC 7884 clinical isolates and *E. coli* ATCC 8739, *E. coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 6633, *Proteus mirabilis* ATCC 2593, *Klebsiella pneumoniae* ATCC 13883 and *Candida albicans* ATCC 10231 museum strains were used as the study objects. The selected strains are representatives of various groups of microorganisms differing in the structure of cell membrane and defense mechanisms.

Monocultures were treated with an air plasma jet generated by a glow discharge at atmospheric pressure and direct current, at a distance of 4 cm between the anode and the microorganisms for 1, 5, 10, and 20 minutes. The discharge current was set to 35 mA, the air flow was 5 L/ min, the temperature of the plasma jet was continuously monitored using FLIR E4 thermal imager and did not exceed 30 °C.

It was established that the pronounced antimicrobial effect of plasma irradiation with respect to monocultures of bacteria appeared only after 5 minutes of exposure. *Ps. aeruginosa* ATCC 15442 museum strain was the most resistant, i.e. the zone of growth inhibition after 10 minutes of exposure was only 6.8%. At the same time, *Ps. aeruginosa* 7884, *Bacillus subtilis* ATCC 6633 and *Kl. pneumoniae* ATCC 13883 strains showed the lowest resistance, i.e. after 10 min of plasma treatment decontamination of bacteria occurred in 35.4 %, 45.9 and 36.0 % of the plate area, respectively [34].

The bactericidal properties of the negative-corona

low-temperature argon plasma were studied for effects on the vegetative form of E. coli strains. E.coli cultures were grown on enriched agar medium in the form of a lawn. Experimental coupons with bacteria were exposed to argon plasma, with an exposure time of 30 seconds to 5 minutes [35]. The data obtained show that the treatment of the plates with plasma jets of a low-current spark for 30 seconds leads to the death of almost all microorganisms within a radius of 1.5 cm. The determination of the inactivation ability of argon plasma by the method of counting colonies shows that after one-minute treatment, only single colonies remain. However, the inactivation zone is not limited to the diameter of the generator nozzle, within which plasma jets of a low-current spark are formed.

Using the above data, the changes in the CFU number during the treatment of packaged dietary supplements with the low-temperature plasma were investigated. The results showed a decrease in CFU by three orders.

Research results allow to conclude that food treatment with low-temperature plasma may be one of the effective ways to reduce the growth of microorganisms, thereby increasing the shelf life and suitability without the need to maintain low-temperature conditions.

5. The use of plasma technology in the production of meat products and to ensure their shelf life

Recently, the plasma treatment method was tested in meat and meat products including pork [36], ham [37,38,39,40], ground meat [41], sausages [42], etc. Studies have shown that atmospheric-pressure cold plasma treatment on the surface of meat products leads to the inactivation of food pathogens improving the safety and stability of meat products while increasing their shelf life. It is also effective to prevent the use of nitrite in their production.

Oehmigen et al. [43] found that plasma does not interact directly with microorganisms in a liquid, but the plasma-liquid interaction leads to liquid acidification in combination with the formation of reactive oxygen species and reactive nitrogen species including nitrate (NO^-_3) and nitrite (NO^-_2), which inactivate microorganisms.

Based on this study, it was suggested that water with nitrites is formed during plasma treatment and may be used as a source of nitrites in meat products. Therefore, the aim of the study was to compare the quality of the emulsion sausages made with plasma-treated water (PTW), celery powder containing nitrite, and synthetic sodium nitrite at a concentration of 70 mg/kg and to evaluate the suitability of PTW as a source of nitrite in the production of meat products [42].

The results of the study did not show a noticeable effect on the change in color characteristics and peroxide value of sausages with PTW during storage for 28 days at 4 °C compared to samples with celery powder and sodium nitrite. Throughout the storage period, sausages with the addition of PTW had a lower concentration of residual nitrite compared to samples made with the addition of celery powder and sodium nitrite. The sensory properties of sausages made with PTW and sodium nitrite did not differ, while the sausages with the addition of celery powder received the lowest scores for flavor and consumer acceptability.

It was established [42] that the total number of aerobic bacteria was below the detection limit of 101 CFU/g for all samples on the day of manufacture (0 days) and remained at this level for the next 14 days of storage. The total number of aerobic bacteria increases to 2 CFU/g after 21 days of storage. The addition of nitrite and PTW limited the growth of aerobic bacteria on days 21 and 28 compared to the control sample. On the 28th day of storage, the total number of aerobic bacteria in the samples with plasma-treated water was similar to sausages made with nitrite solution and lower than in sausages with celery powder, although the difference was small. Color characteristics, peroxide value and sensory properties of the samples produced with PTW were similar to those of sausages with sodium nitrite. From the results obtained, it was concluded that PTW might be used as a source of nitrite.

The aim of the work conducted by the scientists of Animal Industry and Biotechnology Department, Chunnam National University, South Korea and Agricultural Biotechnology Department, Center for Food and Bioconvergence was to study the possibility of using water after plasma treatment as a source of nitrite in curing of meat products [41]. After plasma treatment of distilled water for 60 minutes, it contained 50 ppm of nitrite. Consequently, the amount of nitrite added in ground meat by means of PTW was 0.001 %, while in ground meat with sodium nitrite this value reached 0.007 %. In order to assess the effect of PTW on the formation of meat color, various samples of ground meat were made (control - ground meat without added nitrite; PTW sample - ground meat with PTW; SN sample - ground meat with added sodium nitrite). After heat treatment, there was no significant difference in the values of lightness (L*) and yellowness (b*) between treatment groups. Redness (a*) of the heat-treated ground meat with PTW were significantly higher compared to the control group. However, a* values of heat-treated ground meat with PTW were lower than those in samples with sodium nitrite ($P < 0.05$). Thus, it can be concluded that PTW may be used as a source of nitrite in curing of meat products. However, plasma treatment system needs to be improved in order to form a large amount of nitrite in water. In addition, an appropriate classification of PTW is necessary, since PTW is neither a synthetic nor a natural source of nitrite.

Further to these studies, ham was produced with the replacement of nitrite or nitrate by plasma-treated water (PTW). To obtain PTW, distilled water was treated with plasma, after which it was injected into pork tenderloin. Ham cured with PTW (HCP sample) showed a lower

content of aerobic bacteria (log CFU/g) and residual nitrite concentration, as well as a higher a* value compared to ham cured with sodium nitrite solution (HCN sample). There were no significant differences in sensory properties between samples of HCP and HCN [40].

At week 0, HCP samples had a lower total number of aerobic bacteria compared to HCN samples ($p < 0.05$). By the end of the second week of storage, the total number of aerobic bacteria in cured ham was 6.52 log CFU/g for HCP samples and 6.68 log CFU/g for HCP samples, respectively. The results are consistent with the assumptions [44] that the treatment of distilled water with a surface dielectric barrier discharge in atmospheric air has a bactericidal effect on the liquid itself.

The residual content of nitrite in all groups decreased throughout the storage time. In addition, the residual concentration of nitrite in HCN samples during storage was higher compared to HCP samples ($p < 0.05$). The authors noted that the residual nitrite content is important for maintaining the quality of cured meat products during storage. However, many consumers are interested in a lower level of residual nitrite due to the potential relationship of nitrite and nitrosamines with cancer.

Scores of appearance, color, odor, flavor, juiciness, chewiness, off-odor and overall acceptability of HCP and HCN samples revealed no significant differences.

Thus, a number of researchers have concluded that PTW may be used as a possible substitute for synthetic nitrite [40,41] and has an antimicrobial activity [44].

Studies have been conducted on the quality of minced ham cured using atmospheric-pressure plasma (APP) treatment [40]. The minced ham was prepared using sodium nitrite, celery powder, and treated with atmospheric-pressure plasma for 30 and 60 minutes. It was established that the content of nitrite in ground meat increased with an increase in duration of treatment with PAD. The content of nitrite in ground meat after treatment with atmospheric-pressure plasma for 30 and 60 minutes reached 40.42 and 60.50 mg/kg, respectively. The pink color of cured ham was formed with all types of treatment, and no significant difference in the color of cured ham (L*, a*, and b* values) was found when using different sources of nitrite. The sensory properties (flavor, color, odor, texture, general acceptability) of minced ham cured with atmospheric-pressure plasma treatment were similar to the same characteristics of minced ham cured with powdered celery. However, the flavor and general acceptability of minced ham cured with sodium nitrite received significantly lower scores compared to those for minced ham cured with celery powder or APP treatment.

The effect of atmospheric-pressure plasma (APP) treatment on nitrite levels and physicochemical parameters of ground meat models consisting of pork, water, and sodium chloride (80:20:1) was also studied during mixing. For research, a compact atmospheric-pressure plasma treatment system was developed and placed in the upper part of the ground meat mixer. It was established that the plasma treatment gradually increased the temperature of ground meat from 0.2 to 20 °C over 60 minutes. Since the recommended final temperature of ground meat should not exceed 10 to 13 °C, plasma treatment was carried out for 30 minutes. The level of nitrites in ground meat increased with an increase in the duration of plasma treatment, reaching 65.96 mg/kg during treatment for 30 minutes. After the plasma treatment for 30 minutes, the pH of ground meat slightly reduced from 6.0 to 5.92. Ground meat a* values increased significantly with an increase in plasma treatment duration, which is associated with an increase in nitrite levels. The redness value increased from 2.13 ± 0.57 to 7.2 ± 0.3 [45].

In addition, an increase in the antioxidant and antimicrobial activity of flavonoids was reported during treatment with atmospheric-pressure plasma (APP) [46].

The purpose of the work conducted by Jung S. et al. [47] was the study of nitrite level and antimicrobial activity of *Perilla frutescens* extracts after their treatment with atmospheric-pressure dielectric barrier plasma (APP) for their further use in production of meat products. Freeze-dried ethanol extracts of perilla treated with APP for 60 minutes contained 3.74 mg/g nitrite. The control sample (freeze-dried ethanol extracts without APP treatment) did not contain nitrite. The minimum inhibitory concentration (MIC) of APP-treated freeze-dried ethanol extracts for *Clostridium perfringens* was 200 μg/ml. The control sample of perilla extract did not inhibit the growth of *C. perfringens* at MIC in the range of 25 to 1000 μg/ml. The MICs of APP-treated freeze-dried ethanol extracts and controls for *Salmonella typhimurium* were 25 and 50 μg/ml, respectively. Thus, new sources of nitrite with increased antimicrobial activity for use in meat technologies may be obtained from the APP-treated natural vegetable raw materials regardless of their initial nitrite level.

Thus, treatment with atmospheric-pressure plasma may be used in curing process as an alternative to the addition of nitrite. However, currently, it is difficult to give a clear classification of PTW, since it is neither a chemical reagent nor a natural source of nitrites [42]. It was suggested that the plasma treatment is a method of water purification that can remove harmful pollutants in it. In this context, PTW may be classified as purified water, containing nitrite and possessing the properties of natural antimicrobial agents.

P. Benecke et al. conducted an experiment, the purpose of which was to study the antimicrobial effect of cold atmospheric-pressure plasma on the inactivation of microorganisms on mortadella sausage slices during 21 days of storage, using *S. enterica serovar Typhimurium*, *E. coli* and *L. monocytogenes* as indicator microorganisms. The cold atmospheric-pressure plasma used in this study was generated by FlatPlaSter 2.0 (Terraplasma GmbH, Garching, Munich, Germany [U = 18 kV, f = 12.5 kHz, P = 0.5 V/cm2]) [48].

Low operating temperatures combined with a short plasma treatment duration (0, 30, 60, and 120 s) at atmospheric pressure showed different effects on the inactivation of *Salmonella enterica serovar Typhimurium* (S.T.), *Escherichia coli* (E.c.) and *Listeria monocytogenes* (L.m.).

The effectiveness of cold plasma treatment in relation to the inactivation of microorganisms on mortadella sausage samples was limited. The maximum inactivation for *S. enterica serovar Typhimurium* was 0.3 log10, while for *E. coli* and *L. monocytogenes* it remained unchanged. In the first two weeks of storage, the researchers did not observe significant differences in microbial counts for both *L. monocytogenes* and *E. coli*. However, after 21 days of storage, significant differences were found, i.e. 0.33 log10 (*L. monocytogenes*) and 0.63 log10 (*E. coli*) when treating samples with cold atmospheric-pressure plasma for 30 and 120 s. *S. enterica serovar Typhimurium* were more susceptible to treatment with cold atmospheric-pressure plasma compared to other studied bacteria. During storage, sausage samples treated with cold atmospheric-pressure plasma showed lower counts of *S. enterica serovar Typhimurium* compared to untreated control samples during the entire storage period. However, the authors noted that the differences in *S. enterica serovar Typhimurium* counts were quite small. This study confirms that *L. monocytogenes* are gram-positive bacteria and have thicker outer membrane compared to gram-negative *S. enterica serovar Typhimurium* and *E. coli*. Thus, they were less sensitive to treatment with cold atmospheric-pressure plasma. In addition, it was noted that *L. monocytogenes* as psychrophilic bacteria have the advantage of reproduction in a wide temperature range and may possibly grow continuously for 21 days of storage at 4 ± 0.5 °C. Furthermore, a high content of fat (20 %) and protein (13 %) in mortadella sausage slices may also create a barrier against treatment with cold atmospheric-pressure plasma [48].

One of the criteria that may be critical for the effectiveness of cold atmospheric-pressure plasma treatment is the gas used [10]. Some authors [38] consider the N2+O2 mixture to be the most effective for reducing the amount of bacteria on meat surface compared to using only ambient air.

Wang et al. [49] noted that cold plasma treatment of chicken fillets in a modified atmosphere (O2/CO2/N2 = 65/30/5) with an increased oxygen concentration allowed reducing the bacterial load by at least two log CFU/g during 14 days of storage.

Chinese scientists conducted studies concerning the effect of dielectric barrier discharge (DBD) plasma on microbial inactivation and discoloration of the pork loin surface [50].

Treatment duration with dielectric barrier discharge (DBD) plasma of pork loin inside sealed polypropylene trays was 60, 120, and 180 s at 80 kV and room temperature. After plasma treatment, samples were stored at room temperature for 24 hours.

After treatment of pork loin with DBD using gaseous media, i.e. air, 40 % O2-MAP and 60 % O2-MAP, the survival rate of aerobic bacteria decreased by 15.2%, 26.7%, and 32.4% when exposed for 60 s and 26.2 %, 39.4 %, and 45.1 % when exposed for 120 s, respectively. With an increase in treatment duration up to 180 s, the survival rate of aerobic bacteria decreased by 42.5%, 49 %, and 53.2 % when using gaseous media, i.e. air, 40 % O2-MAP and 60 % O2-MAP, respectively.

There were no significant differences in color characteristics between plasma-treated samples regardless of the treatment duration. So, with a higher oxygen concentration of 60 % at treatment duration of 120 s, L* values in pork loin decreased from 51.2 to 50.23. It was determined that a* value is sensitive to oxygen concentration and plasma treatment duration. This is consistent with the findings by Attri et al. [51], who determined that the structure of hemoglobin changed in the presence of particles with high reactivity generated using DBD plasma in the presence of various gases. The authors suggested that DBD plasma also induces a change in the structure of myohemoglobin, which may affect a* values in meat. As for b* values, they significantly increased with increasing oxygen concentration and treatment duration. Similarly, b* values in bacon did not change when helium was used, but increased at higher input power when helium/oxygen was used [16].

The effectiveness of plasma treatment depends on the surface of the treated medium. Joo-Sung Kim et al. [52] studied the effect of atmospheric-pressure plasma using argon on the inactivation of *S. aureus* placed on polystyrene plates, in agar plates and inoculated on the surface of dried beef cut into 5x5 cm pieces, depending on the duration of treatment. It was established that after 2 minutes of treatment, the amount of *S. aureus ATCC 12600* reduced by 3-4 log CFU/g on polystyrene and agar, but on a sample of dried beef, this effect was achieved only after treatment for 10 minutes. This allowed the authors to suggest that surface properties may significantly affect the degree of *S. aureus* inactivation by plasma. No significant changes in fatty acid composition, color and shear force for dried beef samples were found.

Analysis of *S. aureus* morphological changes on the surface of dried beef after the plasma treatment was carried out by scanning electron microscopy and showed that within 5 minutes after treatment, *Staphylococcus aureus* cells were severely damaged with the formation of many holes in membrane. Optical spectrum analysis suggested that reactive oxygen species, especially the singlet oxygen at 777 nm, are mainly responsible for the inactivation and cellular deformation of *S. aureus*.

Bacteria on irregular surface migrate to a depth of approximately 140 microns due to the capillary effect. At these distances from the surface, microorganisms in general are not exposed to the sterilizing treatment.

In addition, ruptures resulting from severe contraction of muscle fibers may provide pathways for bacteria penetration into a depth. All these factors affect the efficiency of sterilization by the dielectric barrier discharge plasma.

Preliminary results of the studies to justify the possibility of using cold argon plasma for sterilizing canned meat and fish products and to evaluate the sterilizing effect of cold argon plasma that were carried out using one-day cultures of *Ps. fluorescens*, *St. aureus*, and *E. coli* with an initial concentration of 5x104 CFU/ml in 50 ml of normal saline [53] showed that plasma treatment for 10 minutes results in almost complete elimination of vegetative cells and spores. Furthermore, and it was established that with plasma-based sterilization, vitamins in canned model vegetable/fish mixtures are better preserved.

The results of the study showed the sterilizing ability of low-temperature plasma for different types of microorganisms and the possibility of its using as an effective method for treatment of meat products to increase their shelf life.

Conclusion

Currently, plasma technologies, despite their great potential, are not available as a sterilizing tool in food industry, mainly due to the lack of studies for the plasma discharge effect on food chemical components, interactions with packaging materials, etc., as well as applicability in industrial production. The use of cold non-thermal plasma in food technology still needs to be thoroughly studied, despite the data obtained on the effectiveness of this type of treatment in the inactivation of food pathogens or natural toxins and, as a result, to increase the shelf life of food products.

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AUTHOR INFORMATION

Nataliya A. Gorbunova - candidate of technical sciences, Scientific secretary, V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, 109316, Moscow, Talalikhina str., 26 . Tel.: +7 -495-676-93-17. E-mail: n.gorbunova@fncps.ru

Contribution

The author completely prepared the manuscript and is responsible for plagiarism

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Review paper

PREREQUISITES FOR THE FORMATION OF D - ENANTIOMERS OF AMINO ACIDS OF ANIMAL PROTEINS IN THE MANUFACTURING PROCESS OF MEAT PRODUCTS

Natal'ya L. Vostrikova*, Oksana A. Kuznetsova, Valentina B. Krylova, Andrey V. Kulikovskii

V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia

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Abstract

The paper presents studies on the presence or formation of d - enantiomers of amino acids in animal tissues or organs, in meat products during its production processes. It is shown that the process of epimerization of L - amino acid residues with the formation of D-enantiomers affect the reduction of the properties of food products, including the formation of oncoassociated subsequent effects on the human body.

Modern control of the quantitative and qualitative composition of d-enantiomers of amino acids in food products, monitoring for stratification of the increased risk of toxic compounds in food are becoming an urgent medical and social problem. The studies planned in this paper are aimed at developing approaches to the creation of food products that reduce the oncogenic alimentary load on human health by solving the problem of technological modification of production, eliminating or minimizing post - translational modifications in proteins that contribute to the formation of d-enantiomers of amino acids. These studies will create a scientific and technological database associated with the risk assessment of carcinogenesis in protein matrices of animal origin. Based on the presented analysis, the task of developing and testing a method to control the accumulation of D-isomers in the course of various technological processes of meat production is extremely popular.

Introduction

There are key aspects and directions in the Strategy of the NTR of the Russian Federation: transition to highly productive and environmentally friendly agro - and water management, development and implementation of systems for the rational use of chemical and biological protection of agricultural plants and animals, storage and efficient processing of agricultural products, the creation of safe and high – quality, including functional, food[1].

Nutrition is the most important element that ensures the maintenance of health, efficiency and creative potential of the nation [2]. Thus in order to reduce the health risks, that are realized in the production of meat products, is necessary to study deeply the nature and depth of post-translational deformations occurring in animal proteins during processing. The study of the processes of epimerization of L-amino acid residues with the formation of D-enantiomers, which affect the properties of food products, including oncoassociated, is one of the most relevant areas of modern biotechnology and biochemistry.

Currently the perspective area of research in the field of determining the composition of finished meat products is the allocation of biomarkers of various components [3,4,5]. Modern control of quantitative and qualitative composition, as well as food safety, monitoring for stratification of increased risk of toxic compounds (formation of carcinogens) in food are becoming an increasingly urgent medical and social problem. The effects of genetic purity, disruption of technological processes, unbalanced formulations, improper storage, transportation and other factors on the chemical, biochemical composition of food is also a major problem of nutrition science.

In this regard, the most important way to ensure the safety and quality of meat products today is the formation of an integrated scientific and technological approach to identify and identify tissue-specific substances of protein-peptide nature, responsible for the formation of various carcinogenic effects and leading to various diseases, including tumors in human cells, with the cumulative effect of consuming a harmful product.

In order to study the carcinogenic effect, it is necessary to study in detail the changes in protein synthesis during the degeneration of the cell into cancer and pay special attention to those proteins that regulate this synthesis. Many proteins and peptides transfer various structural changes as a result of post-translational modifications, especially under the influence of technological operations, primarily affecting enzymatic processes in animal tissues, in the production of products [6].

Recently, in the scientific community of particular

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interest are the optical structures of amino acid isomers. Posttranslational modification (reactions of polypeptide transformation into an active protein) is a covalent chemical modification of a protein after its synthesis on a ribosome. For most proteins, posttranslational modification is the final stage of biosynthesis, which is part of the process that occurs during gene expression. Posttranslational modifications can regulate the duration of proteins existence in the cell, their enzymatic activity and interactions with other proteins [7,8]. Among the covalent posttranslational modifications of peptides synthesized by ribosomes, epimerization of L - amino acid residues with the formation of D-enantiomers takes a special place, the presence of which affects the biological activity of peptides, and also affects the development of cancer, inhibition of flavin enzymes of brain tissue and violation of other metabolic processes associated with amino acids.

Only L-stereoisomers of amino acids are involved in protein synthesis by ribosomes. In natural proteins, D-amino acids are rarely found, as a rule, in the composition of peptide antibiotics, which are synthesized by enzymatic complexes of microorganisms without the involvement of ribosomes. Another source of D-amino acids in proteins may be spontaneous racemization of their L-stereoisomers in polypeptide chains as a result of various processes in protein matrices of animal origin. The amino group (NH2) does not formed in people, therefore, to maintain nitrogen balance, as well as to carry out the biosynthesis of protein compounds, which include amino groups, the body must necessarily receive and assimilate certain amounts of essential and interchangeable amino acids [9,10].

However, not all amino acids from food are available. In some cases, under the influence of hard (thermal) or unusual cooking food proteins acquire special properties, which makes it difficult to dispose of them. In addition, in violation of the functions of the glands of the digestive apparatus, especially with the weakening of the enzymatic activity of digestive juices, which occurs in physiological (in the later stages of ontogenesis) and pathological conditions and diseases, the intensity of the splitting (digestion) of proteins is reduced, the processes of absorption of amino acids are disturbed. In amino acid preparations obtained by acid hydrolysis, the availability of amino acids is also reduced due to the fact that their D-forms are not absorbed by the body. It is possible that d-forms of amino acids are formed during the processing of meat products in an acidic environment, as well as in patients with hyperacid condition. Consequently, in violation of the processes of digestion of protein products and the weakening of the absorption of amino acids, as well as the formation of racemates and D-forms of amino acids, conditions are created to reduce the availability of these metabolites, which generates their deficiency, leads to a violation of amino acid imbalance and significant shifts in nitrogen metabolism. Incomplete utilization of amino acids coming from food adversely affects the biosynthesis of structural proteins, enzymes, hormones, vitamins, mediators and other highly active compounds, the molecules of which include amino acids.

In addition, with functional liver failure caused by age (senile) changes or diseases of the hepatobiliary system, the processes of transamination, deamination of amino acids are weakened or distorted, which exacerbates violations of nitrogen metabolism and contributes to the appearance of amino acid imbalance in the body [9].

The developed topic, aimed at solving the fundamental problem - the design of innovative food products of directed action using the methods of modern applied epigenomics and biomedicine, taking into account the study of the mechanisms of formation in the product of onco-associated substances, will expand approaches to the creation of safe food products based on meat. A significant role in this process is played by food components of different nature. The study of the prerequisites for the formation of carcinogenic load and the determination of post-translational modifications occurring in the protein matrices of animal origin, in the production of products consisting of multi-component protein matrices obtained from raw materials of animal and vegetable origin, are most relevant in the modern study of conformational changes in protein, in the production of functional ingredients actively used in the meat industry.

That confirms the focus of this work in accordance with the direction of the Strategy of the NTR of the Russian Federation, in terms of effective processing of agricultural products, the creation of safe and high-quality, including functional, food.

Research methodology

The studies, planned in this paper, are aimed at developing approaches to technological modification of meat production to exclude or minimize posttranslation processes in proteins that lead to the formation of D - enantiomers of amino acids.

The result of the work will be:

- the development, testing and validation of the method of identification of D-enantiomers of amino acids;
- the recommendations for improving the technological processes of production of products to ensure their safety for the human body.

To achieve the goals set in the work, methods and approaches related to the determination of amino acid composition and its various isomerization forms in protein matrices of animal origin will be integrated. And with the development of methodological solutions for the determination of D-enantiomers of amino acids.

The methodological solutions developed by the authors will be used to identify post-translational modifications occurring in protein matrices of animal origin, including connective tissue proteins, for possible correction of technological modes of meat production. The main methodological solutions implemented in the framework of this research project to perform the

tasks are based on high-performance chromatography with different detection methods (photometric, mass spectrometric) with preliminary derivatization of the analyte. Preliminary study of chromatographic behavior of derivatizing agent - ortho-phthalic aldehyde (OPA) derivatives on reversed-phase sorbents and study of the properties of chiral columns, with the prediction of the order of elution of amino acid derivatives using theoretical calculations and optimization of sample preparation of real objects, will reduce the criteria of reproducibility and precensiveness of the method to not significant relative to the confidence interval of the contents of the component.

Conducting research in this direction will allow to formulate and significantly expand approaches to the creation of safe food by creating a scientific and technological data base associated with the risk assessment of the formation of d-enantiomers of amino acids in protein matrices of animal origin. As part of the work will be identified, studied and systematized epimerization of L - amino acid residues, depending on the technological parameters of the production of the product, as well as an assessment of exposure to d - enantiomers of amino acids with carcinogenic properties, and evaluated their safety during storage of the product.

Discussion

1. Spatial structure of amino acids

The basis of the entire biological system of man, are protein chains, in which the presence of all essential amino acids is crucial. Essential amino acids cannot be synthesized in the human body. Therefore, their intake with food is necessary. Amino acids - a kind of biological atoms of the universe, "privacychoice", from which nature builds the diversity of the surrounding animal and plant microspace. They are formed into topological, sequential chains (polypeptide), which are called proteins, or protein molecules. In the process of digestion, the protein you eat is broken down into amino acid fragments, and then the body collects new protein chains from them, but already such as it needs. Amino acids are derivatives of carboxylic acid invariants, in which one hydrogen atom is replaced by an amino group. Basically, there are two types of amino acids, with the same composition of the substance. They are classified as groups - D and L amino acids. D-form (*deksier-right*) this are right rotating isomers and L – form (*levus-left*) – left rotating isomers. For the first time, the phenomenon of molecular dynamics chirality was discovered by French microbiologist and chemist Louis Pasteur. In modern science, these rotations are called-molecular spin. Both forms have the same composition and topological basis, but differ from each other in the structure and properties of the substance. Their main differences from each other are the opposite, mirror-spatial isomer and chemical structure. In this regard, they can not be combined with each other movement in space. It is like two of our hands, mirror-like, but when we start to combine them finger to finger, the left hand describes a circle to the right, and the right to the left. Right rotating and left rotating amino acids, diametrically, differently act on the same form of life, in whatever form this life was not represented [11].

Today it is well known that in living organisms due to dominate amino acids with left-hand rotation of the L-form. But there are exceptions in nature. If an amino acid is not preceded by the letter L or D, then it does not have a mirror opposite isomer and is represented only in one form. This type of amino acids includes, for example, a replaceable amino acid-glycine. But all, without exception, essential amino acids have opposite isomerism. If we draw the spatial formula D and L of amino acids, we can see that they are identical in composition, but opposite in arrangement of molecules and rotation. Such a phenomenon as the spacial isomer of amino acids is associated with the ability of the molecule to rotate the plane of the polarized light beam in opposite directions. When assembling our body, L-form amino acids are able to affect the D-form amino acids found in plant proteins. This effect occurs with the energy resource of the noradrenaline factor and the redox process. D-isomeric forms of molecules present in the structure of plants, under certain conditions, especially in cellular hypoxia, have a destructive effect on the human body. Scientists today know more than 200 amino acids. But the human body is built only from 21 amino acids, of which 8 are essential and most important for the proper structure of all our cells. Moreover, a prerequisite is that these 8 essential amino acids represent only the left - sided L-form. If the protein chains in the body are built on this principle, then the cell itself will have a left-hand rotation and it will have beta-synthesis processes, which is a natural factor for the life of the human body. If we get essential amino acids from plant food, which have a right-hand rotation-D-form (*deksier-right*), the cells will be built with the right-hand spin, where there will be processes of photosynthesis with an emphasis on oxygen-free existence. This type of cell is almost identical to the cells of plant nature and their body or trying to dispose of accelerated division, or they form colonies in our body, which leads to mutations, and eventually to diseases and tumors. This is an important nuance in the construction of our body, which is often not known to those who make the basis of their nutrition vegetable protein. Complete vegetarianism is possible only if a person has energy practices that allow to activate all the chakras and energies of our spine – Kundalini. But of those who thoughtlessly accept the principles of such nutrition, almost no one owns such practices and all their efforts are limited only to ideological asceticism and self-acceptance, built on the ideas of correctness. Energy practices, many of which are forgotten, allow the body to activate a biophysical resource that is able to twist any form of amino acid into the left spin. In the body of an ordinary person who does not own such practices, this does not happen.

In the normal body, there is only spatial l-correction of the amino acids we obtain from plant foods. This is one of the biophysical functions of our body, which largely depends on the hormonal and electrolyte homeostasis [12,13]. As a result of scientific research at the Pittsburgh Medical University, on the basis of non-invasive methods of cell research, it was found that the cell division of its two halves have a different wave response. This means that cell division is represented by differences in the process of apoptosis and in the topological configuration of protein chains. This is because in the process of life is a constant process of disposal of improperly formed cellular material. If the body is able to disassemble one of these halves to the essential amino acids of the left-hand back and on their basis to produce a new Assembly, it will be a process of longevity. This new understanding of the processes of apoptosis, raises the question of the importance of the essential amino acids of the L-form, in the very first row, when we start to think and talk about the right lifestyle and nutrition. Human nature and nature in General are so arranged that any substance or food carries both benefits and risks. If we talk about our body and nutrition for him, it is necessary to know that the rejection of animal products containing essential amino acids with left-hand rotation, leads to enormous changes in the entire system of cell construction. But human nature is so arranged that animal proteins also carry the risk and disease, if the diet is not balanced. The balance is based on the fact that we have two digestive systems – acid and alkaline. Both of them take different part in the process of life and dangerous long and quantitative shift in one direction or another, when it comes to the choice of animal or plant products. Plant food will not harm the body if it is pre-properly prepared for intake and if the body constantly receives essential amino acids of L-forms. Under the preliminary preparation refers to aerobic salting, watering and fermentation of plant products, resulting in their preliminary fermentation system isomeric correction – spin neutrality. With a lack of essential amino acids left-hand rotation, the body uses not only food, but also what it already has in the form of cell mass. Using what we already have is dismantling our old cells to essential amino acids, which are used again in protein building, to form new cells. And all the other, interchangeable amino acids remaining from the old cells, the body utilizes through our excretory systems. As a rule, young organisms cope better with this work. It is for this reason that many diseases begin in adults. The whole system of construction of living systems is determined by the astrophysical guide of nature, which manifests itself in the biophysical primacy of fields and energies that are foremen in the construction and functioning of the body. American biologists from the Goddard Center, who participated in the research program of the phenomenon of life on Earth, published an analytical article in the scientific journal Proceedings of National Academy of Sciences, which explains the reasons for the dominance in organisms of living beings amino acids left-hand rotation, which became the basis of life on earth living beings. But nature left options, when in animal body these processes can be disturbed by. That is, in the animal world, cells can be built on the principles of photosynthesis using D-amino acids, which nature uses to build the plant world. In these cases, the constructed living system is incomplete for its synthesis of the group, as it begins mutational transformation (rebirth). Therefore, the conflict of two forms of astro-biophysical processes begins in the body, which leads to the destruction of the species determination program [14].

2. Food system

The technologies currently used in the production of protein hydrolysates are carried out by chemical (under the action of mineral acids and alkalis at elevated temperatures) and enzymatic (using proteolytic enzyme preparations) methods. During acid hydrolysis, all types of peptide bonds between different amino acid residues are broken down, and therefore almost complete protein cleavage is possible. However, acid hydrolysis as a technological process has a number of disadvantages (partial or complete decomposition of a number of amino acids, the formation of melanoidins, need to neutralize the acid).

When alkaline hydrolysis of protein occurs racemization of amino acids, resulting in hydrolysate loses biological value. In food production alkaline hydrolysis of protein is not used. The existing methods of obtaining alkaline hydrolysates of proteins are mainly aimed at obtaining preparations for technical (non-food) purposes.

Enzymatic hydrolysis for food enterprises is not economically beneficial. Therefore mainly used chemical under the action of mineral acids and alkalis.

Embedding D-amino acids in the L-polypeptide chain leads to the formation of irregular spatial organization of the molecule, the chain begins to break and change its direction, while changing the orientation of the ligands. The accumulation of D-amino acids in proteins leads to a change in the tertiary and quaternary structure of the protein and, consequently, a decrease in its functional activity. Thus, the appearance of D-amino acids can be self-sufficient factor leading to the formation of abnormal proteins and the development of various diseases, including tumors in human cells, with the cumulative effect of consuming a harmful product.

Modern directions of predictive medicine are aimed at regulating the changes in the activity of genes that are not associated with changes in the DNA itself, which persist steadily in a number of cell divisions. A significant role in this process is played by food components of different nature. Carcinogenic and translational regulatory properties previously identified in food products consisting of multi-component protein matrices obtained from raw materials of animal and plant origin, attract more and more scientists to their study.

In the last 10 years, extensive research has been carried out around the world to study the substances of protein and peptide nature contained in raw meat and finished meat products, as well as formed in the process of various technological processing and in some way determining the quality and functional characteristics, as well as the safety of finished food, however, a comprehensive study of the mechanisms of formation of D - enantiomers is not covered.

The current work in the claimed area is mainly aimed at the study of post-translational modification of D-amino acids in peptides, the study of the role of D-amino acids in the pathogenesis of various diseases, the study of conformational changes in D-amino acids. A large number of works are carried out in farm areas on the influence of amino acid enantiomers in medicines [15].

D-isomeric forms of molecules, under certain conditions, especially in cellular hypoxia, have a destructive effect on the human body. If we get essential amino acids from food, which have a right-hand rotation - (D - form), the cells will be built with the right-hand spin, which will be the processes of photosynthesis with an emphasis on oxygen-free existence. This type of cell is almost identical to the cells of plant nature and their body or trying to dispose of accelerated division, or they form colonies in our body, which leads to mutations, and eventually to diseases and tumors. American biologists from the Goddard Center, who participated in the research program of the phenomenon of life on Earth, published an analytical article in the scientific journal Proceedings of National Academy of Sciences, which explains the reasons for the dominance in organisms of living beings amino acids left-hand rotation, which became the basis of life on earth living beings. But nature gave options, when in animal body these processes can be disturbed by. That is, in the animal world, cells can be built on the principles of photosynthesis using D-amino acids, which nature uses to build the plant world.

The first data on the presence of D-amino acids in animal tissues were obtained in 1950. Free D-alanine was isolated from the blood of some insects by chromatography [16]. Later, the presence of D - amino acids such as D-alanine, D-phenylalanine, D-glutamate, D-ornithine, D-serine, D-asparagine, D-methionine and D-cysteine was revealed in the composition of polypeptides in animals [16,17,18,19,20]. It was suggested that D - amino acids in mammals appeared from the products of endogenous flora or spontaneous racemization of L-amino acids in the structure of polypeptides during aging [21]).

Studies have shown that D-aspartic acid was found in various tissues of the body, such as the lens [22,23], the brain[24,25,26], as well as teeth, skin, bones, aorta, erythrocytes, lungs and ligaments in aging [23]. D-serine is defined in β-amyloid in Alzheimer's disease [25,26].

In certain diseases in the body starts a conflict between two forms of astro-biophysical processes, which leads to the destruction of the program determination. The most characteristic diseases of this group are various tumor processes and partly the processes of blood disease [27,28].

Conclusion

The results of numerous studies show that the epimerization of L-amino acids with their transition to D-form plays the main role in the aging of the body, and aspartic acid is most susceptible to racemization. Racemization accelerates the action of ultraviolet radiation. The accumulation of D-amino acids in proteins leads to a change in the tertiary and quaternary structure of the protein and, consequently, a decrease in its functional activity [29].

The particular interest are also studies aimed at determining the D-isomers of amino acids in food and medicines, and in living matter.

Currently, the following methods can be used to determine the level of D - amino acids [30]:

1 Nuclear-magnetic resonance spectroscopy - is a method that allows to determine the ratio of stereoisomers in solution, as well as to identify individual stereoisomers.

2 Determination of optical activity - the method is carried out using a polarimeter device. A stream of light is passed through the solution and the sign of rotation of the polarized light (right-turning or left-turning) will indicate the absolute configuration of the compound.

3 The diffraction analysis - allows to estimate the absolute configuration of the crystalline product.

4 Immunohistochemistry - is a method based on the binding of an antigen to an antibody.

Usually amino acids are determined by high-performance liquid chromatography (HPLC). There is a direct method for determining amino acids and their optical isomers on chiral stationary phases and a method for determining derivatized amino acids used for the analysis of complex objects. O-phthalic aldehyde (OFA) is often used as a modifying agent in conjunction with various nucleophilic reagents. Derivatization with this reagent occurs in 3-5 minutes, the derivatives formed are easily determined by the method of reversed-phase HPLC with a fluorometric detector [31]. The chromatographic behavior of OFA derivatives has been well studied in the reversed-phase HPLC regime, but there is no data on the separation of derivatized amino acid enantiomers on chiral columns. This is due to the decline in the selectivity of the column due to the interaction of OFA with the functional groups of the sorbent [32]. A promising solution to this problem is the use of HPLC with mass-spectrometric detection.

Multi-stage technology of production of the finished product (fine grinding, Ambassador, heat treatment, formulation) and multi-composition formulations make it difficult to guarantee the output of the product, which would not contain protein conformations.

To date, the provision of high-grade protein and the absence of dangerous contaminants in the finished product, especially provoking allergic reactions, exacerbation of chronic diseases and beer-producing risk of oncological

formations, has become one of the main areas of medicine and veterinary medicine, and methods for the study of these accumulations are actively introduced into the control sphere.

In General, the presented information about the leading groups working in this thematic area, the results of the most successful developments, as well as the general bibliometric analysis reflect the high degree of demand for these studies and indicate the correct choice of subjects of the proposed study. World laboratories are actively developing methodological solutions for the study of epimerization of amino acids, which can be combined with the proposed developments and improve their efficiency [33,34]. At the same time, the study of the formation of D-isomers in raw materials for food production and modification of technological solutions that lead to their reduction by other groups of researchers is not conducted, which ensures the fundamental novelty of the proposed work.

Based on the above analysis, the task of developing and testing a method to control the accumulation of D-isomers in the course of various technological processes of meat production is extremely popular.

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AUTHOR INFORMATION

Natal'ya L. Vostrikova – candidate of technical sciences, head of laboratory «Scientific and methodical work, biological and analytical research», V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences. 109316, Moscow, Talalikhina str., 26 . Tel.: +7-495-676-79-81. E-mail: vostrikova@fncps.ru. *corresponding author

Oksana A. Kuznetsova - doctor of technical sciences, director, V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences. 109316, Moscow, Talalikhina str., 26. Tel.: +7-495-676-72-11. E-mail: o.kuznecova@fncps.ru

Valentina B. Krylova - doctor of technical sciences, professor, chief researcher of the Department of technological developments, V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences. 109316, Moscow, Talalikhina str., 26 . Tel.: +7-495-676-74-01. E-mail: v.krylova @fncps.ru

Andrey V. Kulikovskii - candidate of technical sciences, a head chromatography laboratory, leading scientific worker of the Laboratory «Scientific and methodical work, biological and analytical research», V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences. 109316, Moscow, Talalikhina str., 26. Tel.: +-495-676-60-11. E-mail: a.kulikovskii@fncps.ru

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