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THEORY AND PRACTICE OF MEAT PROCESSING

ТЕОРИЯ И ПРАКТИКА ПЕРЕРАБОТКИ МЯСА

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Original scientific paper

RISK ANALYSIS AND IDENTIFICATION OF CRITICAL CONTROL POINTS (CCP) IN PRODUCTION OF NATURAL INTESTINAL CASINGS

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Key words: *safety management system, HACCP, risks, risk analysis, critical control points, CCP, packaging, casings, natural casings, intestinal casings, meat products*

Abstract

Implementation of the HACCP system in enterprises manufacturing natural intestinal casings is topical for ensuring high quality and confidence in safety of manufactured products. The paper examines hazardous factors in production of natural intestinal casings, analyzes risks with assessment of the probability of hazardous factor occurrence. To this end, a Pareto chart was constructed, unacceptable risks were determined, CCPs were revealed using the decision tree, critical control limits were established for each CCP and the requirements for their monitoring were specified. In addition, the paper gives risk analysis for the stage «salting» with expert assessment of the severity of consequences from realization of a certain hazardous factor and the probability of this realization for each risk. The risk of microbial growth due to addition of the insufficient amount of salt was classified as a biological factor with the severity of consequences 3 and the probability of realization 3. The risk of microbial growth due to violation of the temperature-humidity conditions and duration of holding on a site is also a biological factor with the severity of consequences 3 and the probability of realization 2. These stages are assigned to CCPs. Using the Pareto chart, the factors that had the highest effect on safety and quality of natural intestinal casings were grouped; with that, the percentage ratio of the revealed hazardous factors was established: biological/chemical/physical/allergens 65/20/15/0 for the whole technological process.

Introduction

Casings perform an important function in all types of sausage products; it is casings that give them a shape and also protect from an impact of an environment. Natural intestinal raw materials have been used for many centuries. Their protein composition is close to the meat composition and, therefore, they withstand the same technological regimes of processing as minced meat, obtain strength under the impact of smoke and hot air, and ensure stability of a finished meat product to microbial action [1].

It is worth noting that natural casings are exposed to the microbiological risk to a larger degree than, for example, polyamide casings as they are a favorable environment for the development of microorganisms. In this connection, the preference is increasingly given to artificial analogues [2].

At present, for production of different types and names of sausage products, manufacturers use beef, hog and sheep small intestine, beef bung, beef middle, beef fatend, hog bladder, hog middle, sheep bung [3].

Currently, the system used in the Russian Federation for the control of materials that are applied as packages for foods is harmonized with the international requirements and ensures their safety for population upon correct exploitation, adherence to the requirements of labeling and storage conditions. However, there is a problem of norming the safety indicators for natural casings due to the lack of a unified legislative document. As the regulative documentation for intestinal raw materials was absent for a long time, the Gorbatov Research Center for Food Systems developed in 2016 the interstate standards GOST 33791–2016 «Pig's casings and bladders. Specifications», GOST 33790–2016 «Bovine's casings and bladders. Specifications», GOST 34107–2017 «Sheep's and goat's casings. Specifications», which give classification of casings, their characteristics, requirements for materials and raw materials for production of intestinal casings, requirements for packaging and labeling, acceptance rules, methods for control and rules for transportation and storage [4].

The majority of manufacturers know that unsatisfactory quality of used casings and risks in the supply chain have an adverse effect on product quality characteristics, which as a result, negatively affect the economic component of any enterprise. Therefore, to ensure complete safety for an ultimate consumer, it is necessary to examine risks not only in the process of sausage production but also at the stages of preparation of casings to be used.

The process of risk management showed itself to be effective in many industrial branches. In the food industry, risk management was brought under regulation in 2013 with coming into force of the Technical Regulation TR CU 021/2011 «On food safety», according to which a manufacturer has to develop, implement and maintain procedures based on the HACCP principles in implementing processes of food production associated with safety requirements for such products [5].

In this connection, implementation of the HACCP system is also topical for enterprises that produce natural

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casings as it allows ensuring predicted high quality and confidence in safety of manufactured products, timely identifying causes of occurrence of non-conformity during the production process and predicting following steps to prevent them in the future.

Materials and methods

Taking into consideration importance of effective risk analysis, in 2018 the V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences carried out studies on safety and quality management for natural casings in the framework of model risk analysis. The objects of the research were hog small intestine as well as the system of management of hazardous factors in production of natural intestinal casings.

Within the framework of the conducted studies, at the first stage of work, the provisions of the HACCP system were implemented, including:

— the hazardous factors typical for production of natural intestinal casings were revealed and described successively for each stage of the technological process;
— risk analysis was carried out — the probability of occurrence and realization of hazardous factors in the production process, as well as severity of consequences of their realization for an ultimate consumer were assessed;
— a Pareto chart was built to reveal the main hazardous factors that affect product safety;
— cause and effect analysis of realization of the biological hazardous factor was carried out by the method of Ishikawa diagram building;
— unacceptable risks that affect safety and quality of finished products were determined;
— CCPs were revealed using the method of the decision tree; for each CCP, the critical control limits were established;
— requirements for CCP monitoring were established.

Analysis and identification of the unacceptable risk were carried out for each potential hazardous factor with consideration for the probability of its occurrence and severity of consequences. To this end, for each hazardous factor, an expert comparative assessment of severity of consequences from realization of this factor and the probability of this event was performed using designations (Figure 1).

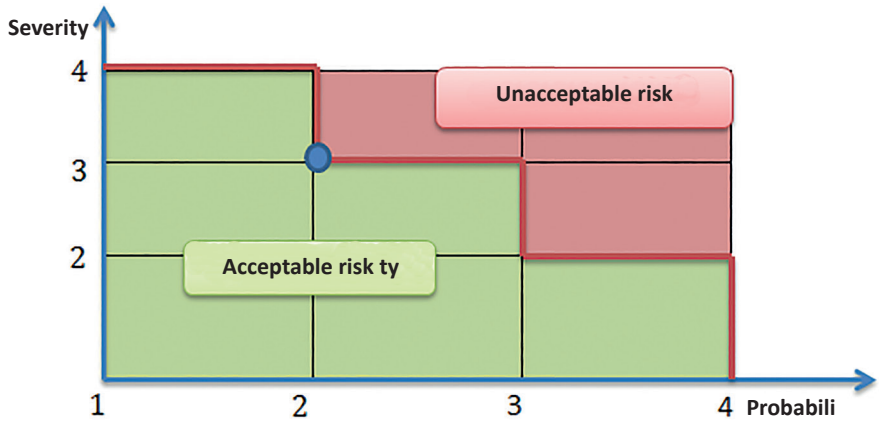
For CCP identification, the method of the decision tree, which is an undirected connected graph with no cycles, was used. The vertexes of this graph are tested hypotheses (assumptions) and «leaves» are possible decisions. Along the path from the root of the «tree» to «leaves», the sequential correction of a decision takes place. To simplify the procedure of building a decision tree and working with it in practice (including for implementation of the research data), only binary «trees» are used; with that, each of the questions linked with vertexes of the «tree» allows only two possible answers — YES and NO [6].

General requirements for the method of the decision tree are established by regulatory documents, in particular, GOST R 51705.1. During research, this method was used with considerations for peculiarities of the production process.

Results and discussion

All stages of the production process were successively analyzed according to the technological scheme presented in Figure 2 taking into account risks that were assigned to the category of unacceptable risks — the zone of the high and medium risk. With that, an effect of the following stages of the production process was taking into consideration regarding the probability of risk realization.

During the research, hazardous factors of manufacturing the natural intestinal casing (hog small intestine) were assessed. Table 1 presents analysis of risks for the stage «salting»; for each risk, an expert comparative assessment of severity of consequences was made with regard to real-



Severity of consequences	Score	Probability of realization
minor ailment that did not lead to serious disorders	1	unlikely (possible once a year)
temporal deterioration of health that did not lead to hospitalization	2	very seldom (once a month)
serious deterioration of health that required hospitalization	3	seldom (once a month)
serious deterioration of health that led to long-term disability or death	4	quite often (once a week)

Figure 1. Diagram of risk analysis

ization of this hazardous factor and the probability of this realization. Only those hazards were taken into account that were on the border and in the area of the unacceptable risk.

The HACCP system distinguishes four types of hazardous factors: biological (B) — microorganisms (including their toxins), viruses and parasites; chemical (C) — chemical substances of natural origin or incorporated into a product during technological processing, physical (Ph) — the presence in a finished product of materials that should not be there and allergens (A).

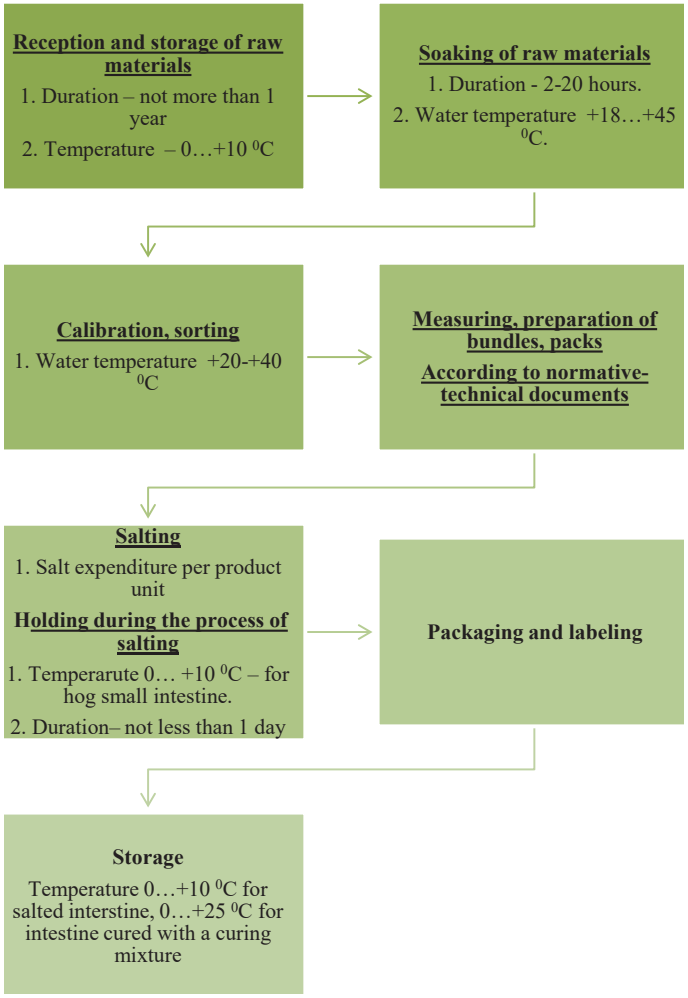


Figure 2. Block diagram for production of hog small intestine

Then, the Pareto chart was built (Figure 3), which made it possible to clearly reveal and assess the main hazardous factors that had the highest effects on safety and quality of natural intestinal casings.

The determinative advantage of the Pareto chart is the fact that it allows ungrouping factors into significant (occurring most frequently) and insignificant (occurring relatively seldom). The Pareto chart shows in the descending order a relative effect of each cause on the general problem.

As a result of classification of risks that affect safety and quality of finished products, it was found that the highest weight had the biological hazardous factor, namely the microbial growth due to violation of the temperature-humid conditions and duration of the presence on the site (26 %)

Table 1. Analysis of hazardous factors

A stage of production process	Description of a hazardous factor	Type of a hazardous factor	Name of a hazardous factor	Severity of consequences	Probability of realization	Potential CCP
Salting and holding of finished products during the process of salting	Microbial growth due to addition of the insufficient amount of salt	B	QMAFAnM, CFU/g coliforms. E.coli	3	3	Yes
	Microbial growth due to violation of temperature-humid conditions and duration of the presence on the site	B	QMAFAnM, CFU/g coliforms. E.coli Salmonella. Proteus. L.monocytogenes, molds, CFU/g, Yeasts, CFU/g	3	2	Yes
	Microbial contamination from personnel, containers, appliances	B	QMAFAnM, CFU/g coliforms	3	1	No
	Use of citric acid	C	Citric acid E330 is used in preservation of casings, it is not a food allergen according to Codex Alimentarius	2	1	No
	Contamination with residues of washing and disinfecting agents from containers	C	For production hygiene, washing agents and disinfectants with burning and irritating action are used to wash food equipment, containers, dressing tables, tools, floors and walls Upon insufficient rinsing of surfaces, the agents can enter raw materials, auxiliary materials and products	2	2	No
	Contamination with foreign bodies from personnel, containers, appliances	Ph	Foreign bodies, insects, rodents, dust, personal items of personnel	1	2	No
	Not detected	A		—	—	

as the used raw materials is unstable in storage as well as upon violation of technological processes and can be a source of human alimentary poisoning.

The conducted investigations allowed determining the ratio of hazardous factors for each chosen stage of natural casing production, which is presented in Figure 4.

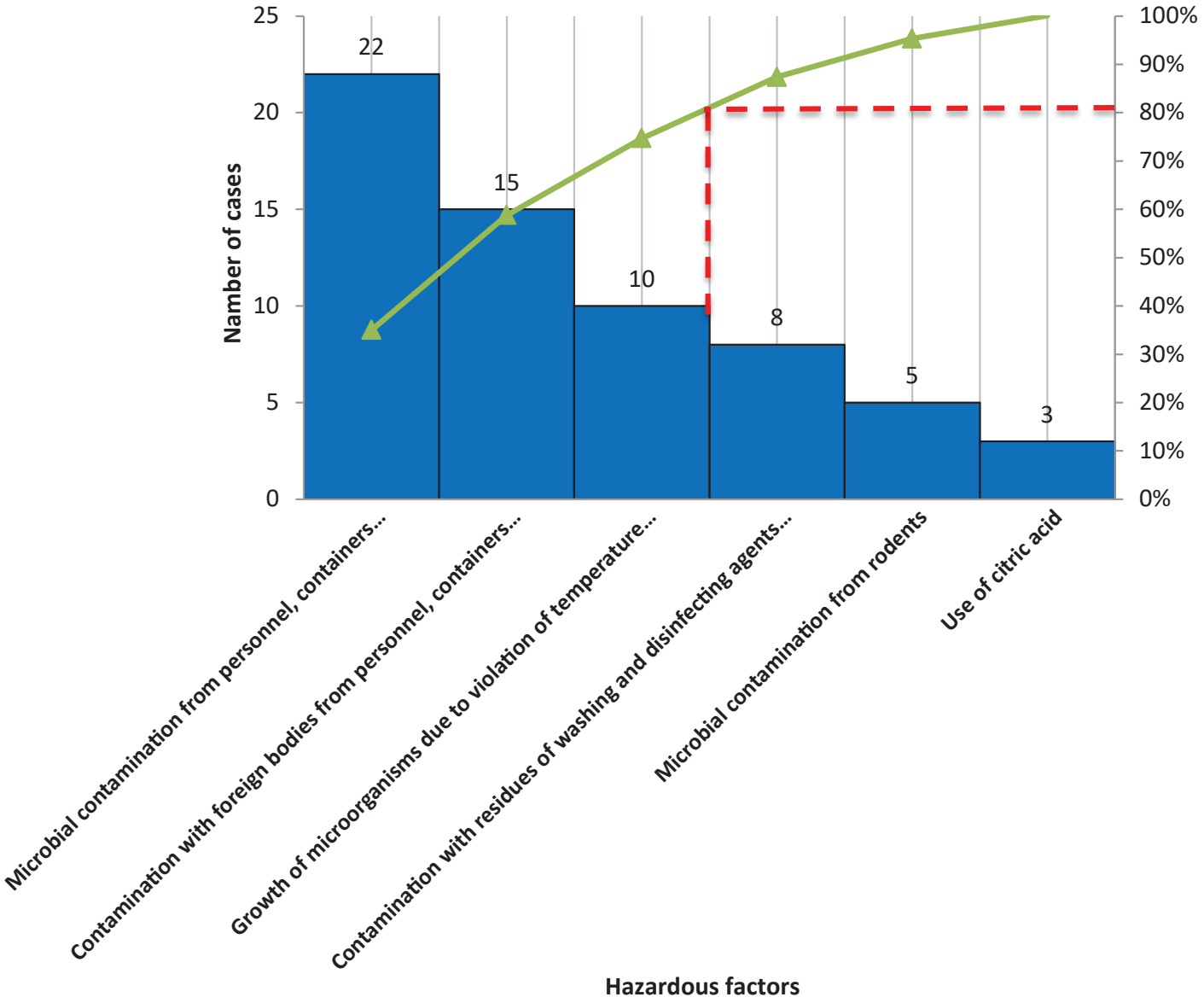


Figure 3. Pareto chart for manufacturing natural intestinal casings

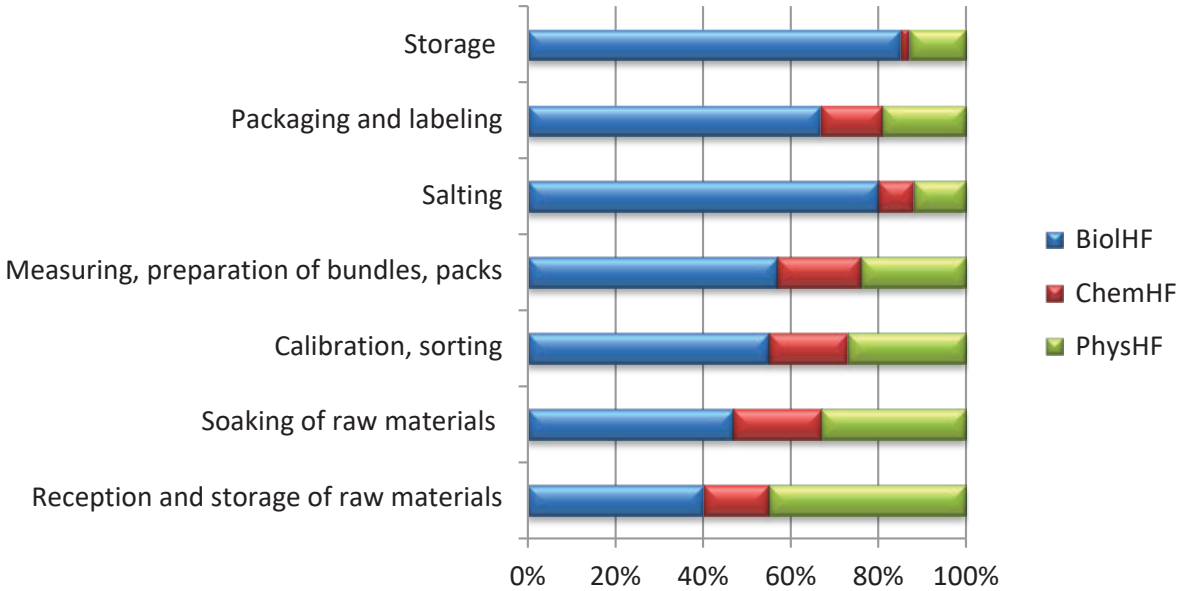


Figure 4. The ratio of the biological (BiolHF), chemical (ChemHF) and physical (PhysHZ) hazardous factors at different stages of manufacturing natural intestinal casings

It is evident that there is a reduction in the proportion of the physical hazardous process from 45 % at the stage of the incoming control to 13 % at the stage of finished product storage. A significant level at the stage of reception and sorting of intestine sets can be explained by the high risk of supply to an enterprise of contaminated raw materials, with package defects or foreign impurities, which suggests carelessness of suppliers and the necessity to revise the procedures of their assessment.

The proportion of the biological hazardous factors increases along the technological process and achieves maximum at the stages of salting and storage of the finished product, which can be explained by an increased risk of the growth of harmful microorganisms due to violation of storage conditions, shelf-life duration and package integrity.

A percentage of the chemical hazardous factors remains to be almost the same along the technological process and is on average 14 %.

It is worth noting that the physical factors in production of casings are the least hazardous as the finished intestinal casings are not the final food products but are used for the following production of meat products. When preparing casings for filling with sausage meat, foreign bodies that accidentally entered finished intestinal casings are removed and, therefore, the risk of their entry into a finished product consumed by humans is practically excluded. In addition, when adhering to the Good Hygiene Practice along with the use of hermetically closed containers, the probability of insect and rodent entry into a finished product is practically reduced to zero.

Using the obtained results, the percentage ratio of the revealed hazardous factors was established: biological/ chemical/ physical/ allergens 65/20/15/0 for the whole technological process.

Analysis of the literature sources [7,8] allows making a conclusion that in the process of organization of work on risk management, an important stage is also determination of the relation of the quality and safety indicator(s) with all possible causes that lead to non-conformity and identification of an effect of causes at all levels of the production process.

One of the effective tools of quality control is the method of the Ishikawa diagram, which helps to study not only the factors influencing the studied object, but also the cause and effect relationships of these factors [9].

The results of the performed cause and effect analysis of realization of the biological hazardous factor in production of the natural sausage casing (hog small intestine) by the method of the Ishikawa diagram are presented in Figure 5.

It was established in the process of Ishikawa diagram studying that personnel of an enterprise plays the largest role in the realization of the biological hazardous factor. Violation of the rules of personal hygiene and production sanitary leads to an increase in the probability of occurrence of this hazardous factor type in the finished product.

To minimize this risk, producers of natural casings should not only plan but also verify training of each employee contacting with a product regarding the rules of personal hygiene, washing and disinfection of equipment, apparatus and surfaces.

The most objective method to achieve this result is to organize a program of occupational training by training courses, instructions and so on. It is also necessary to develop and adhere to clear rules of behavior during disease of employees and visitors. The following cause in terms of impact is maintenance of the necessary production environment. In an enterprise manufacturing natural casings as well as in a food enterprise, it is necessary to pay attention to maintenance of the specified temperature-humid conditions, especially in the rooms for storage of raw materials and finished products, which violation leads to the growth of undesirable microorganisms.

An absence or insufficient use of the bactericidal lamps in the production process, which operation time should be strictly controlled and recorded by a responsible person in the corresponding registration document, also results in the risk of the growth of undesirable microflora [10].

When analyzing the causes of the realization of the chemical hazardous factor, it was found that the most significant are personnel and the technological parameters of equipment.

In analysis of hazardous factors, it is necessary to take into consideration the control procedures as arising problems can be prevented using permitted and non-toxic washing agents for disinfection of equipment, containers, appliances with corresponding supporting documents (certificates of conformity, instructions and so on) in all cases where it is possible. It is also necessary to remember about corresponding personnel training, control of disinfection procedures, control of equipment after cleaning.

A lubricant that is used for maintenance of equipment operation and is intended to be in contact with a product should have a class that envisages this use.

If we draw a parallel to the process of production of artificial packages for meat products (for example, trays for semi-prepared products), then in analysis of a chemical hazard, it is necessary to take into consideration that a technology of production contemplates addition of acetaldehyde, formaldehyde, ethyl acetate, lubricating materials, light stabilizers, antioxidants, solvents (hydrocarbons, alcohols, glycol ethers, ketones and esters) and other chemical compounds that can lead to a risk of chemical migration into a food product [11,12].

Therefore, in case of existence of this threat, for its prevention in production, it is necessary to introduce effective control measures, which can include timely equipment maintenance, adherence to the specified recipe, instruction of personnel and keeping corresponding documentation.

Dyes used for printing of packages should not contain hazardous substances, which can penetrate into the finished products that have a favorable wet environment.

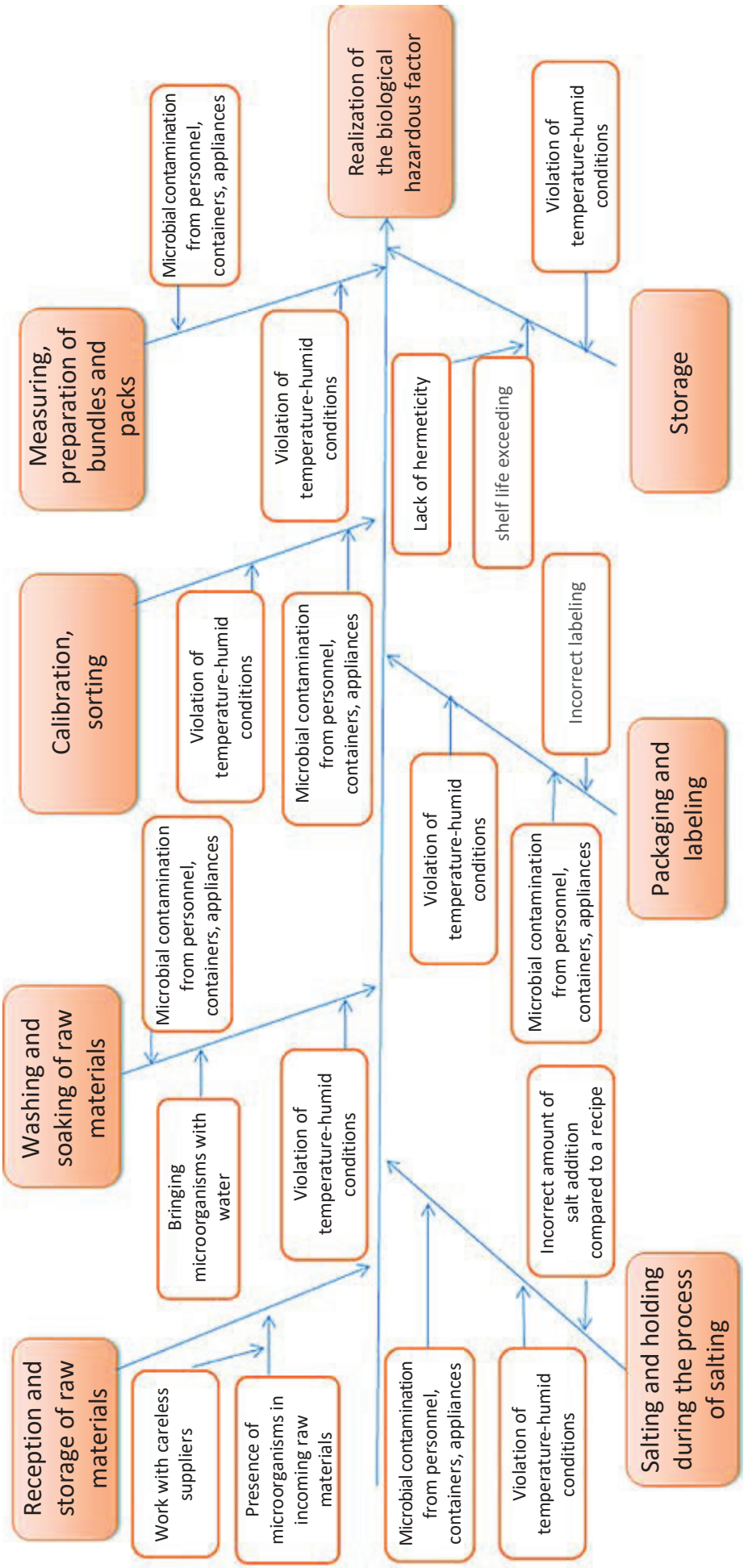


Figure 5. Cause and effect analysis of realization of the biological hazardous factors in production of natural casings

Correspondingly, if multi-layer films are used, all layers should be safe for a product and prevent surface contamination with hazardous substances.

When analyzing causes for realization of the physical hazardous factor, it was established that the physical hazard in the finished product can emerge from several sources such as inappropriate auxiliary facilities and equipment, production environment, employees upon violation of corresponding instructions, which eventually can lead to manufacture of unsafe meat products upon insufficient control by a supplier of casings and a manufacturer [10].

When studying a risk, it is also necessary to take into account a risk of incorrect package labeling, which is often underestimated by manufacturers as attention is largely paid to product safety at the stage of its production.

Meanwhile, inaccurate labeling is often a main cause of product recall as, not infrequently, producers forget to provide information, for example, about a presence of potential allergens in a product or use an incorrect font size. Manufacturers of labeled natural casings should carry out planned risk assessment and use control methods to prevent a risk of mixing labels or incorrectly labeled materials.

As a result of the performed analysis, preventive actions that are presented in Table 2 were developed.

Then, based on the revealed potentially hazardous factors, a critical control point (salting of finished intestinal casings) was identified using the decision tree (Table 3).

For revealed critical control points, the methods and frequency of hazardous factor control were developed, the controlling parameters were determined. The production monitoring and control actions are presented in Table 4.

To ensure safety of a product being at a stage identified as a critical control point, the critical limits of controlled indicators presented in Table 5 were established.

The final stage of the work was determination of corrective actions in case of departure of the process from the established critical limits (Table 6).

The questions of risk management in the food industry were studied by a sufficient number of foreign and national scientists. In Europe, the problems of safety and quality of food products began to be actively discussed already in the 1930th and this question is still topical today — in the age

Table 2. Preventive actions

Stages of production process	Description of hazardous factor	Type of hazardous factor	Preventive action
Salting and holding of finished products during the process of salting	Microbial growth due to addition of insufficient amount of salt	B	Control of salt amount according to the specified recipe Instruction of personnel
	Microbial growth due to violation of temperature-humid conditions and duration of the presence on the site	B	Control of temperature-humid conditions 3 times per shift Exclusion of holding of products before salting
	Microbial contamination from personnel, containers, appliances	B	1. Adherence to sanitary rules and norms by personnel
	Use of citric acid	C	1. Adherence to the established concentration according to a recipe 2. Control of accompanying documentation on citric acid during incoming control
	Contamination with residues of washing and disinfecting agents from containers	C	Control of container washing quality Control of the concentration of a washing agent Instruction of personnel
	Contamination with foreign bodies from personnel, containers, appliances	Ph	Visual examination. Observation of production sanitary Provision of personnel with work clothes adherence to personal hygiene rules by personnel
	Not detected	A	—

Table 3. Detection of critical control points

Stage or operation of production process	1. Is there control at this stage of the production process? YES/NO	1a. Whether control is necessary at this stage of the production process YES/NO	2. Whether this stage of the production process was developed specifically to eliminate or reduce a hazardous factor? YES/NO	3. Can a hazardous factor realize (emerge or increase) at this stage? YES/NO	4. Can the next step eliminate a revealed hazardous factor or reduce a possibility of its occurrence to the acceptable level? YES/NO	Critical Control point YES/NO	No. CCP
Salting and holding of the finished product during the process of salting							
Microbial growth due to addition of the insufficient amount of salt	YES	—	NO	YES	NO	YES	1
Microbial growth due to violation of the temperature-humid conditions and duration of the presence on the site	YES	—	NO	YES	NO	YES	1

Table 4. A list of critical control points

Critical control point	Number of CCP	Hazardous factor	Controlled parameter	Production monitoring		Control action
				frequency	Control method	
Salting and holding of the finished products during the process of salting	1	Microbial growth due to addition of the insufficient amount of salt	Compliance with the recipe: salt expenditure per a casing bundle	Once per shift	Instrumental/measuring salt expenditure	Daily calculation of salt expenditure by a technologist, recording of monitoring data in the»Journal of salt expenditure» control»
		Microbial growth due to violation of the temperature-humid conditions and duration of the presence on the site	Time from the moment of control until salting of the finished products	Upon accumulation of casing lots of the same name, size and category of quality	Instrumental/ time specifications	Control of duration of casing accumulation for salting at the site
			holding temperature for beef small intestine	Twice a day	Instrumental/ Measuring temperature and humidity using a portable thermometer and humidity meter	Monitoring recordings of a thermometer and humidity meter by a shop foreman; recording of monitoring data in a check list of control for temperature and humidity conditions

Table 5. The list of critical limits

No.	Operation	Number of CCP	Hazardous factor taken into account	Controlled parameters	Critical limits
1	Salting and holding of finished products during the process of salting	1	Biological: Microbial growth due to addition of the insufficient amount of salt	Salt expenditure per product unit	Not less: Hog small intestine — 0.55 kg/bundle
			Biological: Microbial growth due to violation of the temperature-humid conditions and duration of presence on the site	1. Duration of accumulation of hog small intestine before salting 2. temperature in the holding room 3. Humidity in the holding room 4. duration of holding	Not more than 3 hours 0…+10oC 60–90% Not less than 24 hours

Table 6. The list of the typical corrective actions

No.	Name and No. of CCP	Controlled parameter	Possible non-compliance	Corrective actions	Person responsible for corrective actions
1	Salting and holding of finished products during the process of salting CCP No. 1	Salt expenditure	Reduction of salt expenditure lower than the value established by a recipe	1. To identify production per shift of finished casings salted with reduced salt expenditure 2. To perform additional salting of casings in a package	shop manager packer
		Temperature (humidity) in the holding room for hog small intestine	Temperature lower than 0oC and higher than +10 °C. Humidity lower than 60% and higher than 95%.	1.To check verification of a thermometer and/or humidity meter 2. To check work of the refrigerator and ventilation system in the room	chief electrician
		Duration of holding during the process of salting	Duration of holding less than 24 hours (for hog small intestine) Duration of holding more than 3 days	1.To carry out visual examination, assess quality of salting and organoleptic indicators of casings. 2. When revealing non-compliance, register products with non-conformance in the journal of product registration and send to additional treatment (secondary processing, washing, additional salting and so on)	Technologist Chief technologist

of the struggle with zoonoses (salmonellosis, listeriosis), study of contaminants (mycotoxins, heavy metals), residuals of pesticides and accidental radioactive contamination.

In the opinion of G. Morgan, identification of risks for humans can often be difficult as in the case of antibiotic resistance. These problems arise due to the complex character of the technological chain of food production and can be revealed only in monitoring of each link of this chain from the perspective of hygiene, presence of contaminants and GMO, as well as other factors [13].

In France, there are several state agencies that are engaged in investigations and assessment regarding the questions of food safety. One of them is the French National Institute for Agricultural Research (INRA). The main activity of INRA is the study of the questions of microbiology and hygiene for optimization of the role of beneficial microorganisms and mitigation of the effect of hazardous microorganisms. With that, however, after analysis of its functions in the field of sanitary surveillance, prediction and expertise, INRA began to pay primary attention to the development of the risk analysis activities and provision of the process of decision making. The Institute also decided to increase by 40% its resources allocated for research of the problems of human nutrition and association between food and health in the nearest four years [14,15].

In 2013, Codex Alimentarius Commission called for division of the functions of risk assessment and their management to ensure independence and transparency of this highly professional type of scientific and technical support.

According to Khamidulina Kh.Kh., food safety assurance nowadays requires attention to all links of the production chain: from primary production operations (including veterinary and animal protection) and feedstuff production up to supply to the ultimate consumer. Food safety can be influenced by any element including environment, from which a corresponding product came [16].

It is also necessary to note an opinion of B. Pakbin who believes that in the risk management system of the food industry, the main risk factors (in addition to biological, chemical and physical) should also include price risk, reputation, brand damage and product recall as the economic risks are no less important for the processing industry [17].

Unfortunately, insufficient attention is paid to the question of risk management in production of natural casings. In our work, therefore, we carried out complex assessment of hazardous factors with detalization of causes for their occurrence. The results of the investigations were taken as a basis when creating a HACCP plan.

Conclusions

The research carried out in the Gorbatov Research Center for Food Systems showed that about 45% of non-compliance in enterprises that produce natural casings occur due to employees' inobservance and lack of knowledge of instructions or insufficient information from management. On this basis, it can be concluded that construction of an effective educational system and motivation of employees is a necessary stage for effective function of the risk management system.

In general, safety of natural casings is ensured by a complex of requirements for materials contacting with food, sanitary-hygienic indicators and physical impurities. It is necessary to note that these requirements apply to all package types regardless of the material used for its production (natural, metallic, polymer, cardboard, glass or from combined materials) [18].

At present, to confirm the above mentioned safety requirements, many large meat processing enterprises conduct audit of its suppliers, for whom the obligatory requirement for obtaining a status of the «approved supplier» is the certified HACCP-based food safety management system in an enterprise, which is one of the confirmations of real safety of used casings.

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Original scientific paper

TECHNOLOGICAL INNOVATIONS FOR TREATMENT OF CASINGS

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Key words: *casings, microbial contamination, treatment, technological aids, antimicrobial efficiency*

Abstract

The article presents the results of substantiation of composition of technological aids for treatment of casings, which provides an increase in microbiological safety of the finished product. The study objects were samples of casings with signs of microbial spoilage, provided by the manufacturer, and prototypes of technological aids for treatment of casings obtained in VNIIPD. Parameters studied: microbial contamination of casings; titratable acidity, active acidity, density of test samples of the technological aids, surface tension and efficiency of aqueous solutions of the samples. Microbial contamination was determined using methods generally accepted in veterinary and sanitary examination; titratable acidity — titrimetric method; active acidity (pH) — potentiometric method; density — densimetric method; surface tension — ring method (du Noüy method). It was found that the surface microbial contamination of defective intestinal membranes includes the following microorganisms: Bacillus spp., Enterococcus spp. and Micrococcus spp. Among the microorganisms the most resistant to various types of treatment are Bacillus spp. microorganisms. It was established, that the samples of the tested synthesized prototypes of technological aids with most effective antimicrobial action were samples based on lactate-, acetate- and propionate-containing additives, including polyhexamethylene guanidine hydrochloride and alkyldimethylbenzylammonium chloride. It was shown, that test prototypes 9, 19, and 28, which include polyhexamethyleneguanidine hydrochloride, alkyldimethylbenzylammonium chloride and polyvinylpyrrolidone, have rather high antagonistic activity against test cultures of L. monocitogenes, E. coli, St. aureus, Sal. typhimurium with the folowing concentration — (600 × 10^6 CFU/ml). Minimum effective concentrations of solutions of technological aids for treatment of casings vary from 0,6 % up to 2,5 %. It was established that minimum effective concentration of solutions of technological aids for treatment of casings corresponds to concentration of the solution with maximum adsorption of the resulting complexes on the surface of the liquid-air phase boundary. Treatment of defective casings with aqueous solutions of the agents with concentrations of 2.5 % for 30 minutes provides suppression of growth of all detected spoilage microorganisms, including Bacillus spp.

1. Introduction

From the very beginning of development of industrial production of sausages, the casings have been used in production of boiled, smoked or liver sausages, wieners, thick wieners and other products. One of the main advantages of natural casings is the ability to shrink at heat treatment of sausages. At the same time, the casings are perishable products that can not be subjected to thermal preservation.

The task of preservation of high quality of raw casings and natural sausage casings for a sufficiently long storage time is one of most technologically important. In the world and domestic practice casings are preserved, mainly, with salting, which leads to a change in the species of their microbial contamination. Microflora of salted casings mainly consists of salt-resistant microorganisms. When stored in uncontrolled temperature conditions, salty casings are affected by red spots and rot [1,2]. In addition it can also be affected with verocytotoxigenic *Escherichia coli* and antibiotic-resistant microorganisms *Salmonella and Enterococciis, Campylobacter spp is possible., Listeria monocytogenes and Enterobacter sakazakit* [3]. In this regard, studies aimed at creating effective technological aids for treatment of casings are relevant.

Commonly used preservatives for improvement of storage capacity of casings are curing mixtures, including acetic, tartaric, lactic and citric acids, their salts and phosphates, sodium salt of dehydracetic acid or sodium salt of dehydracetic acid with sodium benzoate or sodium sorbate, or with sodium propionate [4,5]. In order to increase softness and elasticity of the casings, treatment of raw casings is carried out using a mixture of pepsin with lactic acid [6]. In order to increase antimicrobial and antioxidant protection of the casings we have offered a composition of a curing mixture containing sodium salt of dehydracetic acid or a mixture of a sodium salt of dehydracetic acid with polyvinylpyrrolidone, a salt of edible acid and dihydroquercetin [7]. We have established an efficiency of a preparation, which contains sodium salt of dehydracetic acid against all the microorganisms most frequently encountered in the meat processing industry (gram-negative bacilli of the following geni: *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, actinomycetes, fungi of *Pénicillium*, *Aspergillus*, *Mucor* geni, and *Torulopsis* yeasts) [8]. Antimicrobial compositions, including food acids, salts of edible acids and compounds of polyguanidine, also suppress vital activity of microorganisms due to formation of monomolecular protective polymer film on the treated surface [9,10,11].

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Currently, a number of compositions are known, including alkyldimethylbenzylammonium chloride, which belongs to the 3rd class of danger, as an active antimicrobial substance. In order to reduce toxicity of Quaternary ammonium bases, the compositions with polymers are created [12].

Based on the results of patent information search for materials about preservation and microbiological safety of casings, it was stated that solution to these problems can be achieved using antimicrobial agents containing edible organic acids, their salts, polymeric compounds and salts of Quaternary ammonium bases.

Purpose of the work: conducting researches aimed at development of prototypes of technological aids for treatment of casings and selection of the most effective of them for application in production of natural sausage casings.

2. Materials and methods

The study objects were:

— samples of casings (pork and beef rounds) with signs of microbial spoilage (with defects) and without them, provided by the manufacturer of natural sausage casings takenfrom four batches;

— test prototypes of technological aids for treatment of the casings, obtained in the course of experimental studies, 29 pieces, liquid in appearance, with different composition and physical-chemical parameters, stated in the Table 1.

Experimental tests were carried out using laboratory equipment of VNIIPD and methods commonly used in practice, in accordance with current regulatory and technical documentation.

Analyzed parameters: sensitivity of the microorganisms to antibacterial drugs, titratable acidity, active acidity and density of test samples of technological aids and surface tension of aqueous solutions of samples of the agents.

The following parameters were evaluated: sensitivity of microorganisms to antibacterial medicines using standard methods (disk diffusion and serial dilution) according to procedural guidelines MUK 4.2.1890–04; titratable acidity — acid-base titration method; active acidity (pH) — potentiometric method using pH-meter pH–150 MI; density — densimetric method using General purpose hydrometers AON–1 with different ranges of measurement; surface tension — ring method using du Noüy tensiometer, made of platinum ring, vessel with test liquid, table and torsion scale with measurement range from 0 up to 360 c.u. and division value 1 c.u.

Triple studies were carried out, the results were processed using primary statistical analysis method.

3. Results

In the researches aimed at development of technological aids for the purpose of treatment of casings we have used ingredients permitted for use in food products «according to standard operating procedures» in accordance with the «Requirements to safety of food additives, flavorings and technological aids» TR TS 029/2012.

It is considered established that the effect of antimicrobial agents on pathogens of microbial damage of the casings is determined by their effect on protein synthesis, enzyme activity, cell membrane and DNA of microorganisms. The main factor of antimicrobial effect of the agents is violation of integrity of the cell membrane of microorganisms. Achievement of the required technological effect is provided by high solubility of antimicrobial agents in water and their high diffusion capacity.

Table 1 shows the results of determination of physical and chemical parameters of experimental samples of the compositions.

Table 1. Physical and chemical parameters of experimental samples of the agents

Sample No.	Main prescription components of the samples	Active acidity, pH unit	Titratable acidity, degrees	Density, g/cm^3
1	Polyhexamethylene guanidine hydrochloride, 20 % solution	9.9 ± 0.1	0	1.035 ± 0.002
2	Lactic acid Sodium hydroxide Polyhexamethylene guanidine hydrochloride Acetic acid Propionic acid Formic acid Sulfamic acid Oxalic acid Ascorbic acid	3.0 ± 0.1	87 ± 1	1.296 ± 0.002
3	Lactic acid Polyhexamethylene guanidine hydrochloride Acetic acid Propionic acid Formic acid Sulfamic acid Oxalic acid Ascorbic acid	0.9 ± 0.1	130 ± 1	1.057 ± 0.002

Sample No.	Main prescription components of the samples	Active acidity, pH unit	Titratable acidity, degrees	Density, g/cm^3
4	Sodium acetate Sodium citrate Sodium metabisulfite Sulfamic acid Oxalic acid Ascorbic acid	pH of 1 % solution 5.9 ± 0.1	195 ± 1	Solid form
5	Sodium lactate Sodium acetate Acetic acid Propionic acid	5.9 ± 0.1	45 ± 1	1.306 ± 0.002
6	Polyhexamethylene guanidine hydrochloride Alkyldimethylbenzylammonium chloride	9.9 ± 0.1	0	1.034 ± 0.002
7	Lactic acid Acetic acid Propionic acid Formic acid Sulfamic acid Oxalic acid Ascorbic acid Polyhexamethylene guanidine hydrochloride Alkyldimethylbenzylammonium chloride	0.6 ± 0.1	250 ± 1	1.063 ± 0.002
8	Glycerol Polyhexamethylene guanidine hydrochloride Alkyldimethylbenzylammonium chloride	9.7 ± 0.1	—	1.055 ± 0.002
9	Sodium lactate Lactic acid Acetic acid Propionic acid Polyhexamethylene guanidine hydrochloride Alkyldimethylbenzylammonium chloride	5.0 ± 0.1	151 ± 1	1.278 ± 0.002
10	Sodium lactate Lactic acid Acetic acid Propionic acid	4.1 ± 0.1	322 ± 1	1.260 ± 0.002
11		4.5 ± 0.1	251 ± 1	1.275 ± 0.002
12		5.0 ± 0.1	146 ± 1	1.287 ± 0.002
13		5.5 ± 0.1	71 ± 1	1.297 ± 0.002
14		5.9 ± 0.1	34 ± 1	1.301 ± 0.002
15	Sodium lactate Lactic acid Acetic acid Propionic acid Polyhexamethylene guanidine hydrochloride	4.1 ± 0.1	305 ± 1	1.257 ± 0.002
16		4.3 ± 0.1	315 ± 1	1.266 ± 0.002
17		4.5 ± 0.1	248 ± 1	1.272 ± 0.002
18		4.5 ± 0.1	250 ± 1	1.274 ± 0.002
19		5.0 ± 0.1	157 ± 1	1.288 ± 0.002
21		4,4 ± 0.1	206 ± 1	1.231 ± 0.002
22		4.8 ± 0.1	121 ± 1	1.240 ± 0.002
23		5.3 ± 0.1	58 ± 1	1.250 ± 0.002
24		5.7 ± 0.1	28 ± 1	1.255 ± 0.002
25	Lactic acid Acetic acid Propionic acid Alkyldimethylbenzylammonium chloride	4.1 ± 0.1	321 ± 1	1.259 ± 0.002
		4.5 ± 0.1	250 ± 1	1.272 ± 0.002
26		5.0 ± 0,1	144 ± 1	1.286 ± 0.002
27	Sodium lactate Lactic acid Acetic acid Propionic acid Polyvinylpyrrolidone Alkyldimethylbenzylammonium chloride	4.0 ± 0.1	263 ± 1	1.229 ± 0.002
28		4.4 ± 0.1	205 ± 1	1,.222 ± 0.0002
29		4.5 ± 0,1	227 ± 1	1.250 ± 0.002
	Sodium lactate Lactic acid Acetic acid Propionic acid Polyvinylpyrrolidone Alkyl dimethyl benzyl ammonium chloride			

Table 2. Evaluation of antimicrobial activity of experimental samples of technological aids in relation to pathogens of microbial spoilage of casings

Sample No.	Acidity		Concentration of aqueous solution of the agent, %	Type of causative agent of microbial spoilage of the raw casings		
	titratable, degrees	active, pH units		Bacillus spp.	Enterococcus spp.	Micrococcus spp.
1	0	9.9	5	+	–	–
			2.5	+	+	+
5	200	5.8	5	+	+	+
			2.5	+	+	+
2	170	4.9	5	+	–	–
			2.5	+	+	+
3	90	3.1	10	+	+	+
			5	+	+	+
			2.5	+	+	+
4	130	0.9	10	+	–	–
			5	+	–	–
			2.5	+	+	+

Table 3. Evaluation of antimicrobial activity of experimental samples of technological aids in relation to pathogens of microbial spoilage of casings

Sample No.	Concentration of aqueous solution of the agent, %	Type of microorganisms isolated from defective casings		
		Bacillus spp.	Enterococcus spp.	Micrococcus spp.
9	5	—	—	—
	2.5	—	—	—
28	5	—	—	—
	2.5	—	—	—

Determination of total microbial contamination of washes of the casings with signs of microbial spoilage showed that washes have significant microbial contamination. Microflora of the surface of the casings consists of cultures of *Bacillus spp., Enterococcus spp. and Micrococcus spp.* geni.

Table 2 shows the results reflecting the effect of the composition, titratable and active acidity of the technological aids prototypes and concentration of their aqueous solutions on effectiveness of antimicrobial treatment of the casings.

For the purpose of increase in antagonistic activity of technological aids, especially in relation to cultures of the genus

Bacillus. *Bacillus spp.*, experimental samples including polyhexamethylene guanidine hydrochloride and alkyldimethylbenzylammonium chloride were synthesized. Physical and chemical parameters of the listed prototypes with introduced antimicrobial substances are shown in Table 1.

Microbiological studies have shown that the tested samples inhibit the growth of enterococcal cultures *Enterococcus spp.* and *Micrococcus spp.* with a significant slowdown in the growth of cultures of the Bacillus genus — *Bacillus spp.* At the same time, it was noted that some samples worsened the consumer properties of casings, making them «dry».

For the purpose of elimination of «dryness» of the treated surface we have prepared prototypes which physical and chemical parameters are significantly different from the previous prototypes, including polyvinylpyrrolidone (Table 1).

Antimicrobial activity of the tested samples in relation to *Enterococcus spp., Micrococcus spp. and Bacillus spp.* cultures was studied, they showed their effectiveness, which is reflected in the table 3 for the samples 9 and 28.

Positive results of evaluation of antimicrobial effect of experimental samples 9 and 28, including polyhexamethylene guanidine hydrochloride, alkyldimethylbenzylammonium chloride and polyvinylpyrrolidone, on pathogens of microbial spoilage of the casings are confirmed by the changes in the surface activity of these samples in comparison with their basis (sample 12), shown in Figure 1.

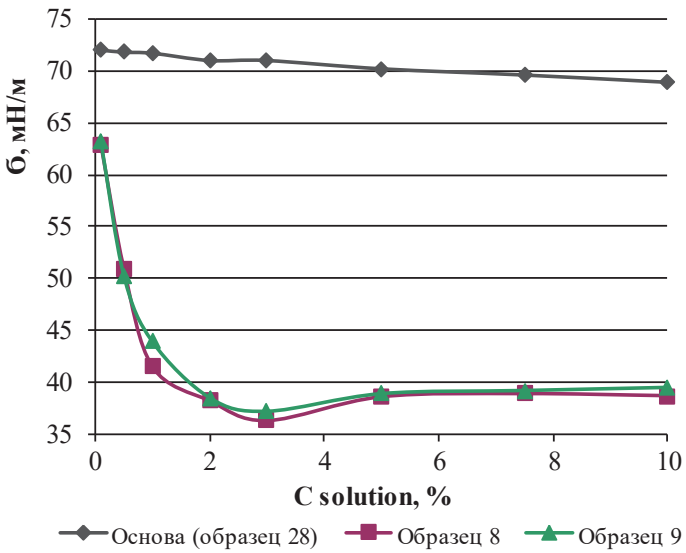

Figure 1. Change of surface tension (σ, mN/m) of aqueous solutions of technilogical aid at the liquid-air phase boundary depends on its concentration (C solution, %)

4. Discussion

Conducted studies of microbial contamination of fresh raw casings have found significant contamination (from several thousand up to tens of thousands and more microbial cells per 1 g). The data obtained are consistent with the known data that the primary treatment of raw casings using traditional technology provides only partial removal (from 65 up to 70 %) of the original microflora.

As a result of the multi-faceted bacteriological studies we have identified microorganisms which most often occupy the surface of the defective casings: *Bacillus spp*., *Enterococcus spp*. and *Micrococcus spp.* It was found that the microorganisms most resistant to various types of treatment are *Bacillus spp.*

At preparation of prototypes of technological aids for treatment of the casings we used ingredients with antimicrobial action. The ingredients in the samples tested include sodium metabisulfite E223, E236 formic acid, acetic acid E260, sodium acetate E262, lactic acid E270, propionic acid E280, ascorbic acid E300, citric acid E330, sodium citrate food 3-substituted 2-water E331(iii), glycerin E422, sulfamic acid, oxalic acid, polyhexamethylene guanidine hydrochloride and alkyldimethylbenzylammonium chloride.

As a result of comparative studies, it was found that the tested prototypes with the most effective antimicrobial action were samples based on lactate, acetate and propionate-containing additives, including polyhexamethylene guanidine hydrochloride, alkyldimethylbenzylammonium chloride and polyvinylpyrrolidone. Treatment of defective casings with aqueous solutions of experimental samples of the agents with concentrations of 2,5 % to 5,0 % for 30 minutes provides suppression of growth of all detected spoilage microorganisms, including *Bacillus spp*.

In order to identify a possible mechanism of action of the tested prototypes of the agents for treatment of casings, an assessment of their surface activity was carried out. We have found synergetic reduction of the surface tension of aqueous solutions of the prototypes of the agents in comparison with the characteristics of the surface tension of their base. The data obtained are indicative of formation in the process of synthesis of experimental samples of complexes of cationic high-molecular and surfactants with anions of lactic, acetic and propionic acids, contributing to increase of their antimicrobial efficiency.

As a result of theoretical and experimental studies we have substantiated a list of technological aids for treatment of casings, which provides a significant increase in microbiological safety of the finished products.

5. Conclusion

Significant microbial contamination of defective casings includes the following microorganisms: *Bacillus spp.*, *Enterococcus spp.* and *Micrococcus spp*. Among these microorganisms the most resistant to various types of treatment of raw casings are Bacillus genus microorganisms — *Bacillus spp*.

The samples of the tested synthesized prototypes of technological aids intended for treatment of casings with most effective antimicrobial action were samples based on lactate, acetate and propionate-containing additives, including polyhexamethylene guanidine hydrochloride, alkyldimethylbenzylammonium chloride and polyvinylpyrrolidone.

We have proposed a hypothetical conception of formation in the process of synthesis of complexes of cationic high-molecular substances and surfactants with anions of lactic, acetic and propionic acids, which provide an increase in antimicrobial efficiency of agents for treatment of the casings.

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Original scientific paper

DEVELOPMENT AND USE OF COMPOSITIONS FROM PRODUCTS OF DEEP PROCESSING OF SECONDARY MEAT RAW MATERIALS

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Key words: *deep processing, collagen, protein ingredients, meat products, quality, efficiency*

Abstract

The development of protein ingredients based on the composites from secondary collagen-containing meat raw materials and obtained by the methods of deep processing attracts increasing attention of specialists.

In the presented work, the composite and mass composition of the protein ingredient from hydrolysates of beef hide split, pork skin and blood plasma in a ratio of 45:45:10 was established. The improved amino acid composition of the protein ingredient due to addition of dry blood plasma suggests an increased biological and nutritional value of the developed product. Addition of up to 15 % of the protein ingredient instead of beef in the technology of minced semi-prepared products improves the rheological and organoleptic characteristics of the finished product.

Introduction

The main direction in processing of agricultural raw materials is an increase in efficiency of present-day production, the development of non-waste technologies of animal raw material processing and involvement of the secondary raw material resources into the commercial-economic turnover.

At present, specialists and technologists of the meat industry pay close attention to a search for new methods for processing of secondary raw materials that remain in the meat processing industry for their more effective use in production of high quality meat products.

The rational use of proteins of collagen-containing raw materials makes it possible to solve many questions of meat production, namely: to compensate a deficiency of soluble proteins, to reduce prime cost of the finished products without a decrease in the nutritional and energy value, to increase the yield of the finished product and to stabilize quality with simultaneous reduction of meat raw material expenditure [1].

The main direction in the rational use of collagen-containing raw materials from the slaughter products of the meat industry is their transformation in order to obtain protein ingredients of different fractional compositions for further use in meat product manufacture.

Nowadays, collagen proteins entering meat processing enterprises are presented by an insignificant number of domestic manufacturers. At the territory of the Russian Federation, the protein containing components of foreign manufacturers or their mixtures are mainly sold by the representatives of different manufacturing organizations and, often, the prices on these protein ingredients are much higher than the prices on the domestic products; however, it is necessary to note that functionality of the preparations from the Russian manufacturers in not inferior to the foreign samples. The proportion of import of collagen proteins both as «pure» mono-ingredients and in the composition of complex food additives or the constituents of the brine preparations exceeds 56 % in volume terms [2].

Taking into consideration an increasing demand of the meat industry for the collagen proteins, the negative attitude of consumers towards plant ingredients in meat products and evolving instability of import supply of food ingredients as a result of the economic sanctions, organization of the system approach in the domestic competitive sector of protein production from secondary products seems to be vitally important.

The fundamental trend in the modern consumer market of food development specified by PNST 329–2018 «green standards» is creation of new food ingredients, which demand is oriented towards the ultimate customer. To this end, the ready complex decisions are necessary including problems regarding the rational use of raw material (meat) components and creation of protein ingredients and their mixtures obtained upon secondary raw material processing [3].

Of particular importance in the use of complex food additives under the conditions of present-day production of meat products is the wide application of protein preparations that significantly affect organoleptic indicators (color, taste, aroma of products) and functional-technological, rheological indicators (brine viscosity, finished product yield, consistency, elasticity, bite properties, strength and so on). For example, the systems based on the connective tissue proteins increase hydration of meat proteins, their

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emulsifying capacity or capacity to bind and hold water and fatty raw materials.

Today, the agro-industrial sector of the Russian economy faces a systemic challenge that predetermines the necessity to update and improve scientific-informational, technical and technological base of the agro-industrial complex on the qualitatively new basis, and the necessity to transfer to an innovative type of the development.

The state program of the development of the agro-industrial complex for the period up to 2024, envisages the most rational use of all types of resources of the food industry; a special attention should be paid to processing of secondary raw materials in the sphere of the agricultural complexes. It is also important to note that the specific weight of raw materials of animal origin in prime cost of the finished products achieves about 60 %, which suggests an importance of its commercial use. The expedient use of secondary raw materials of the meat industry is important as it allows solving problems of the development of non-waste ecologically pure and safe technologies in the continuous commercial conditions [1].

Collagen fibers, which form all types of the connective tissue, support general physical and structural integrity of the body and take part in protective, metabolic and receptor functions.

Collagen prevails in derma, cartilages, bones and vessel walls. Collagen constitutes about 30 % of total protein mass in mammals, and cutaneous covering accounts for about 40 % of it [4,5]. Collagens of cutaneous covering contain large concentrations of proline and oxyproline (about 20 % of the sum of all amino acid residues), glycine and alanine (more than 50 % of the sum of all amino acids) [4].

Healthy people, as a rule, do not show a deficiency of nonessential amino acids (proline and oxyproline). For gerontological patients and people with disorders of collagen metabolism of different types, regular intake of amino acids that are contained in collagen at the maximum level is vitally important.

Therefore, investigations aimed at the development of protein ingredients with multicomponent composition and increased amino acid content seems to be topical.

The aim of this work was the development of a protein ingredient obtained from a mixture of collagen containing secondary products of the meat industry and its use in the technology of minced semi-prepared products.

The main task of optimization, as a rule, consists in obtaining full-value formulations in terms of the amino acid composition; that is, no amino acid should be limiting regarding the reference — the «ideal protein» according to the FAO/WHO scale.

Moreover, to develop a protein ingredient, it is necessary to detect its constituent fractions, their percent ratio in a formulation, an amino acid composition, a proportion of a formulation and its effect upon adding into forcemeat systems of meat products. It is also necessary to study properties and indicators of the meat formulation system and products made with its use.

Objects and methods

The formulation of the protein ingredient contained dry blood plasma (Veos, Belgium) and dry hydrolysates of beef hide spit [6], pork skin [7], obtained in the laboratory conditions by nonenzymatic hydrolysis with the protein mass fraction of 97.2 % and moisture of 2.8 %. Hydrolysates were dried in a drying unit with counter-swirling flows of inert bodies [8]. After preparation and comminution of meat raw materials, the protein ingredient (instead of the main raw material — beef) and additional water were introduced during forcemeat mixing. Prepared forcemeat was supplied to the forming equipment and formed patties were frozen to the end medium-volume temperature of –18 °C. Frozen patties were fried and the functional-technological and organoleptic indicators were determined in the finished product after thermal treatment. The protein ingredient with an improved amino acid composition and minced semi-prepared products were produced and the finished product was analyzed in the pilot production shop of the technological center of KT «OOO Stern Ingredients».

The amino acid content in the hydrolyzed split and pork skin was determined after specific sample preparation [9] by the method of reversed-phase HPLC using a liquid chromatograph Shimadsu LC–20 Prominence (Japan) with UV detection (254 nm). The chromatographic column 250x4.6 mm, Supelco C18 5 μm (USA) was used. In calculation of the content of essential amino acids in dry blood plasma and its amino acid score, the data on the amino acid content (in %) to blood protein were used.

The mass fraction of moisture in raw materials and finished products was determined by the method of drying to constant mass [10].

The mass fraction of protein was measured by the Kjeldahl method [11].

The mass fraction of fat in forcemeat was measured by the reflectometric method with α- monobromnaphtalin by the method of VNIITeK, a branch of the Gorbatov Research Center for Food Systems [12].

Moisture holding and moisture binding capacities were determined by the centrifugation method [13].

Organoleptic assessment of the minced semi-prepared product made with different proportions (10, 15, 20 %) of beef replacement with the protein ingredient was carried out by the profile method and assessed by 5-point scale [14]. Twenty respondents took part in the assessment, among them were 12 males and 8 females at the age of 20 to 35 years. All respondents had the technological education and possessed knowledge of meat and meat product technology.

Ultimate strength was measured on the apparatus of the Valenta type by rupturing the surface of the minced semi-

prepared products with changing masses of weights. The parameter was calculated taking into account half of the mass of the total load and a table, g/cm^2:

The modulus of elasticity was determined using the consistometer and calculated by the equation:

$$E = \frac{(M_{gr} + M_{st})g}{S \cdot E},$$

where, M_{gr} — critical mass of the load determining rupture, kg; M_{st} — mass of a table, on which a sample was placed, kg; g — free fall acceleration, m/s^2; S — area of a sample, m^2; $S = \Pi D^2/4$, where D — a diameter of a sample, mm; E — relative deformation; $E = \sigma/H$, where σ — readings of the indicator, mm; H — thickness of a sample, mm. M_{gr} =0.1 kg; M_{st} = 0.027 kg; G = 9.81 m/s^2.

Shear force was measured in a product sample that had a shape of a rectangular parallelepiped 10x10x40 mm. A sample was placed on the platform of the instrument with the long side along the string. The string fixed on the carriage was placed on the product surface (longitudinally). After that, the platform of the carriage was gradually loaded with weights. The mass of weights, at which the string began to cut a product, was fixed. The mass of the carriage (52 g) and a diameter of the cutting string (1 mm) were taken into account upon calculation.

Shear force P (Pa) was determined by the equation:

$$P = \frac{(M + 0.052) \cdot g}{L \cdot D},$$

where, L = 0.05 m — the length of the wire; D = 0.01 m — the diameter of the wire; g = 9,8 м/с^2 — free fall acceleration; M — mass of weights, kg.

Results and discussion

The strong covalent bond is observed in the primary and quaternary structures of collagen. The secondary and tertiary structures are linked by the non-covalent interactions, which are much weaker. In the tissues, collagen fibrils achieve the large size forming collagen fibers, for example in tendons and derma, or homogeneous mass of their microfibrils as in cartilages [4,15]. This peculiarity of collagen structure is necessary to take into account when developing methods for deep processing of secondary raw materials to obtain ingredients with a given composition and properties [6].

The amino acid composition of secondary products is presented by amino acid oxiproline, which is characteristic for the connective tissue, while tryptophan and cystine, which are characteristic of any muscle tissue, are absent. The average content of amino acids in the hydrolysates of secondary products (per 100 g total protein) is shown in Table 1.

It can be seen from the analysis of the values presented in Table 1 that the essential amino acid (EAA) content in pork skin was 14.00 g/100 g protein. This was 1.32 lower than the EAA content in beef split.

Table 1. Amino acid composition of collagen in beef split and pork skin

Amino acid	Content g/100 g protein in pork skin	Content g/100 g protein in beef hide split
Essential amino acids (EAAs):		
Isoleucine	1.46	1.88
Leucine	2.80	3.73
Lysine	2.40	3.95
Methionine + cysteine	1.24	0.97
Phenylalanine + tyrosine	2.40	3.34
Threonine	2.00	2.26
Valine	1.70	2.47
Total:	14.00	18.60
Nonessential amino acids:		
Alanine	4.10	10.32
Arginine	3.88	8.22
Aspartic acid	3.90	6.95
Histidine	1.15	0.70
Glycine	16.71	26.57
Glutamic acid	6.58	11.16
Serine	2.15	4.27
Proline	5.50	12.8
Oxyproline	11.43	12.81
Total:	55.4	93.80

It is also important to note that collagen of beef hide has the high content of proline and hydroxyproline. Hydroxyproline is not found in such high amounts in any other protein except elastin. Proline occurs in collagen, largely, in the sequence glycine-proline-X, where X is often presented by alanine or hydroxyproline. Collagen does not contain cystine or tryptophan, and the presence of oxyproline and oxylysine noticeably differentiates it from other proteins in living organisms (these amino acids do not occur in the composition of other proteins). The role of these amino acids is exceptionally important in stabilization of the triple-helical conformation of the collagen molecules [4,16].

The sum of essential amino acids is about 22 % of all collagen amino acids. However, the mass fraction of methionine, tyrosine and histidine is very low, while tryptophan, cystine and cysteine are absent; therefore, the nutritional value of collagen of surface covering is low as well as of collagens that are constituents of other organs and tissues.

The unique properties of collagen allow developing highly functional protein ingredients that begin to gain the largest popularity in meat product manufacture as protein ingredients of animal origin have high values of moisture holding and fat holding capacities, moisture binding capacity and functions of emulsifying agents, which is very important for production of high quality foods [5] with high indicators of both technological and consumer properties of the finished products.

Each year, the question is raised regarding effective processing of secondary raw materials of the meat process-

ing and agricultural industries, which is directed towards solving the ecological tasks, extending food assortment and increasing the production volumes of domestic protein ingredients and their advancement on the Russian market of additives with the aim of active competition with import production. It is important to note that secondary products (beef tendons, beef hide split and pork skin), which are rich in collagen, are the main source for commercial production of protein ingredients of animal origin. For example, by the end of 2016, the volume of pig husbandry products was about 4,500 thousand tones, and if we consider the yield of skins of about 8% of carcass weight, then the secondary resources will be 360 thousand tones, which should be a driving force for their processing and a new stage in production of domestic stabilizing protein systems for products of economy, middle and premium segments [3].

In designing a fractional composition of the protein ingredient, different ratios of hydrolysates of beef split and pork skin were used. The best variants of ratios were determined by the value of ultimate strength of the forcemeat system, which showed that the maximum values were achieved at a ratio of the hydrolysate formulation from beef split and pork skin of 1:1.

The content of essential amino acids in the hydrolysate formulation and the calculated amino acid score are presented in Table 2.

Table 2. The content of essential amino acids (mg/g protein) in the hydrolysate formulation of beef split and pork skin and its amino acid score

Amino acid	EAA content, m/g protein	FAO/WHO	Amino acid score
Leucine	32.6	70	0.46
Lysine	65.4	55	1.18
Methionine + cystine	23.4	35	0.66
Phenylalanine + tyrosine	33.1	60	0.55
Threonine	21.9	40	0.54
Valine	34.9	50	0.69
Isoleucine	19.3	40	0.48
Tryptophan	—	10	—

However, in the mixture of hydrolysates with the composite ratio of 1:1, the low content of isoleucine, leucine and threonine, and complete absence of cystine and tryptophan was noticed. Therefore, taking into consideration the values of amino acids and their amino acid score, for creation of a protein ingredient, it is necessary to compensate its composition with additional components (for example, dry blood plasma) to increase its biological value.

Based on the amount of essential amino acids (in %) with respect to blood protein [13], their content in dry blood plasma and amino acid score were calculated, which is shown in Table 3.

It can be seen from the analysis of table 3 that dry blood plasma is a source of essential amino acids and their content is higher than the norms for «reference» protein, which are specified by the requirements of FAO/WHO. Therefore, blood plasma can be used for enrichment of the biological value in a formulation with hydrolisates of hide split and pork skin.

Table 3. Content of essential amino acids (mg/g) in dry blood plasma and their amino acid score

Amino acid	Content of EAAs, mg/g protein formed from the sum of albumins, globulins and fibrinogen	Amino acid score
Teucine	108.4	1.54
Lysine	98.8	1.79
Methionine + cystine	52.1	1.49
Phenylalanine + tyrosine	114.3	1.91
Threonine	67.5	1.69
Valine	73.8	1.47
Isoleucine	25.7	0.64
Tryptophan	19.1	1.91

The calculated content of essential amino acids of the protein ingredient and its amino acid score are presented in Table 4.

It can be seen from the data in Table 4, than upon addition of 50 % of dry blood plasma, it was possible to approximate the amino acid content to the «reference» protein and partly compensate for the absence of tryptophan

Table 4. Content of essential amino acids and amino acid score of the protein ingredient of different formulations

Amino acid	Protein ingredient composed of 90 % of hydrolysate mixture and 10 % of dry blood plasma		Protein ingredient composed of 80 % of hydrolysate mixture and 20% of dry blood plasma		Protein ingredient composed of 50 % of hydrolysate mixture and 50 % of dry blood plasma	
	EAA content, m/g protein	Amino acid score	EAA content, m/g protein	Amino acid score	EAA content, m/g protein	Amino acid score
Leucine	40.18	0.57	47.76	0.68	70.50	1.00
Lysine	68.74	1.25	71.08	1.29	82.10	1.49
Methionine + cystine	26.27	0.75	29.14	0.83	37.75	1.08
Phenylalanine + tyrosine	41.22	0.68	49.34	0.82	73.7	1.23
Threonine	26.46	0.66	31.02	0.78	44.7	1.11
Valine	39.79	0.79	42.68	0.85	54.35	1.08
Isoleucine	19.94	0.50	20.58	0.51	22.5	0.56
Tryptophan	1.91	0.19	3.82	0.38	9.55	0.95

and low content of isoleucine, leucine and cysteine in the mixture of the protein ingredient. The further increase in the proportion of dry blood plasma will lead to a significant increase in prime cost of the protein ingredient and finished product.

At the second stage, the functional-technological indicators and structural-mechanical characteristics were studied in the minced semi-prepared products made with addition of the protein ingredient into the forcemeat system to replace 10, 15 and 20 % of meat raw material (beef). The protein ingredient contained meat hydrolysate from beef split, pork skin and blood plasma in a ratio of 45:45:10. A sample that did not contain the protein ingredient was used as the control.

The main raw materials for preparation of the minced semi-prepared product were chilled beef of the first grade under GOST R 52601–2006; chilled trimmed semi-fat pork under GOST R 53221–2008; protein-fat emulsion in a ratio of 1:10:15 (1 — an emulsifying agent, 10 — pork speck and 15 parts of water). Edible salt, edible chicken eggs, black powdered pepper, powdered allspice, rusk flour and food grade phosphates Carnal 2110 (Budenheim) were used as food ingredients. The recipe of the control sample of the semi-prepared products is presented in table 5.

Table 5. Recipe of the semi-prepared product

Raw materials	Amount, kg/100 kg:
Beef of the first grade	40.00
Semi-fat pork (80/20)	25.00
Protein-fat emulsion 1:10:15	12.00
Chicken eggs	1.20
Chopped onion	4.70
Edible salt	1.20
Black powdered pepper	0.15
Powdered allspice	0.05
Rusk flour	3.70
Water/ice	12.00

Investigation of the properties of raw forcemeat for minced semi-prepared products with the added composite mixture was carried out at a temperature of 4°C. The data on the composition, moisture holding capacity (MHC) and forcemeat strength are presented in Table 6.

Table 6. Properties of raw forcemeat for minced semi-prepared products at a temperature of 4 °C

Proportion of replacement of meat raw materials with the composition mixture,%	Components, %			MHC, %	Strength, g/cm²
	moisture	protein	fat		
0 %	71.5	12.5	17.1	63.1	53.0
10 %	70.8	11.7	16.3	64.9	57.5
15 %	70.5	11.5	15.9	65.8	58.4
20 %	69.3	11.0	15.7	67.2	59.1

Analysis of the values of the presented indicators of the control and the experimental samples of the minced semi-prepared products shows that MHC and the forcemeat strength increased correspondingly to a proportion of protein ingredient addition. Due to the higher MHC in the forcemeat samples with replacement of part of meat raw materials, the finished (thermally treated) semi-prepared products had lower frying losses. An increase in moisture of the fried samples of the minced semi-prepared products led to the juicier product.

After thermal treatment, ultimate strength, modulus of elasticity and shear force depending on an amount of meat raw materials replaced with the protein ingredient were measured in the samples of the minced semi-prepared products. The data are presented in Figure 1, Figure 2 and Figure 3.

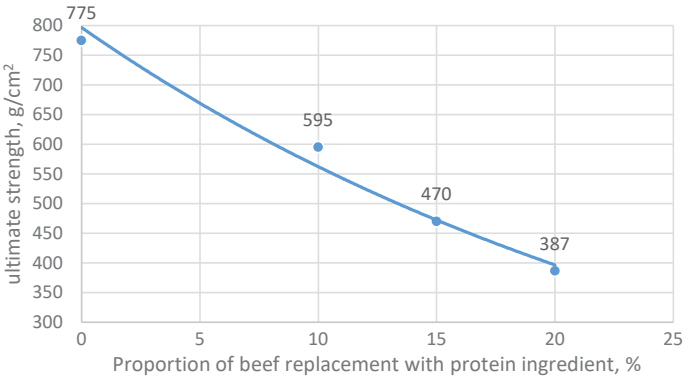

Figure 1. Dependence of ultimate strength in the minced semi-prepared product on the level of beef replacement with the protein ingredient

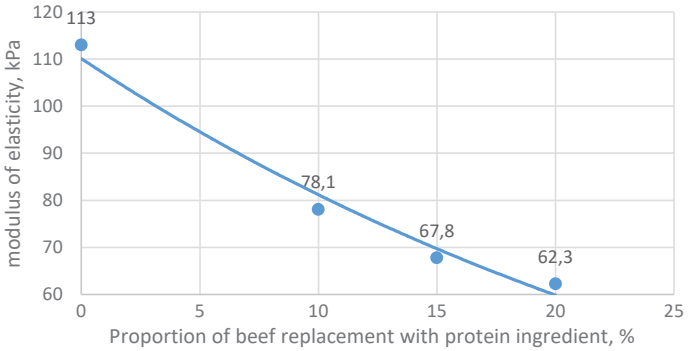

Figure 2. Dependence of modulus of elasticity in the minced semi-prepared product on the level of beef replacement with the protein ingredient

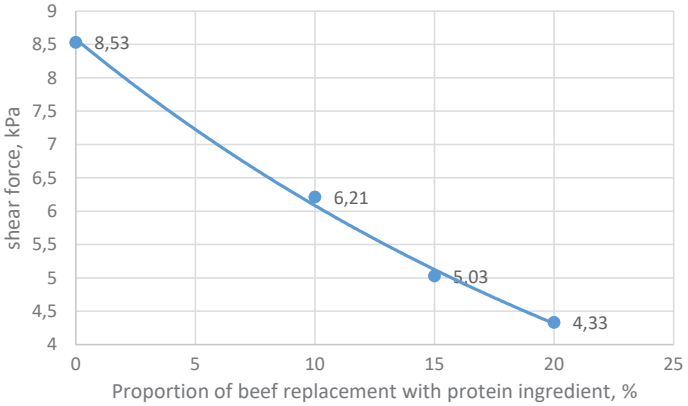

Figure 3. Dependence of shear force in the minced semi-prepared product on the level of beef replacement with the composite mixture

Modulus of elasticity and ultimate strength of the patty samples decreased with an increase in the dose of the added protein ingredient. This is indicative of an increase in tenderness and juiciness of the finished product. However, upon addition of more than 15% of the ingredient instead of beef, the values of modulus of elasticity in the finished product significantly decreased regarding the values of the control samples. A significant increase in looseness and deterioration of patty quality were noticed. When a dose of addition of the protein ingredient increased, the shear force values in the finished product samples reduced; that is, the product became more tender and softer. Upon replacement of more than 15 % of beef with the protein ingredient, the values of shear force in the experimental samples decreased with regard to the values of the control sample; with that, the patties had the crumbly texture.

Organoleptic investigations of the samples are presented in Figure 4. It was also noticed that all samples of the semi-prepared products had odor that was characteristic of the high quality raw materials, an oval shape, a surface without irregular and ruptured edges and homogeneously breaded with wheat rusk flour. On the cut surface of the finished product samples, the homogeneous distribution of well mixed forcemeat and the absence of any lumps of dry carriers were observed; it was noticed that all samples had pleasant aroma of spices. Upon increasing a percent of protein ingredient replacement, consistency of the finished product became crumbly and spreading. The best samples were the finished products with the mass fraction of replacement equal to 15 %. An increase in addition of up to more than 15 % can be considered inexpedient.

Reduction of price of the control recipe for a meat semi-prepared product with maintenance of its functional properties is a priority for a producer.

Figure 5 presents the data on prime costs of the recipes under investigation

Reduction of the recipe price by 7–12 % was achieved upon replacement of 10–20 % of meat raw materials, respectively. This also reduced prime cost of the finished product.

Conclusion

The performed investigations showed that in the protein ingredient with the complex formulation, which contained hydrolysates of beef hide split, pork skin and dry blood plasma in the percent ratio of 45:45:10, an amount of essential amino acids approximated the «reference protein» according to the FAO/WHO norms. This composition has the high biological value and emulsifying abilities due to blood plasma proteins, mainly albumins, and high functional-technological properties and rheological characteristics due to hydrolysates of the mixture.

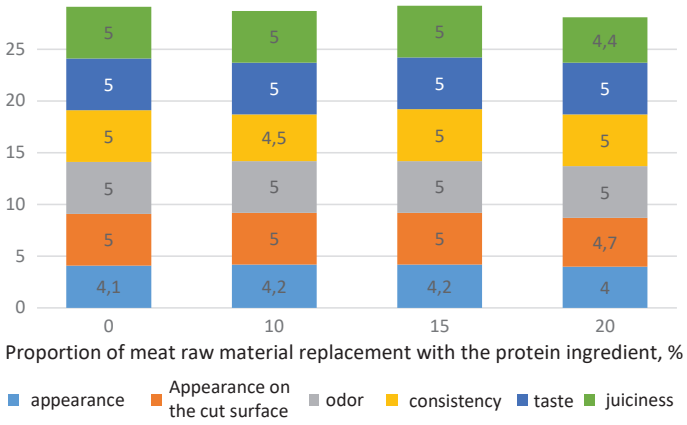

Figure 4. Organoleptic assessment of the minced semi-prepared products with different replacement of meat raw materials with the composite mixture

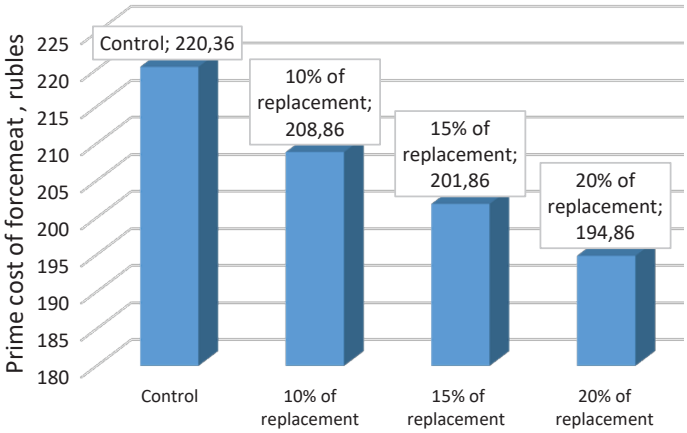

Figure 5. Prime cost of the recipes for patty production with replacement of the main raw materials with the protein ingredient

Analysis of the quality indicators of the minced semi-prepared products made with the use of the composite mixture showed that replacement of 15 % of meat raw materials with the composite mixture was the best; product consistency became more tender and juicier and at the same time less loose compared to the control sample.

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Review paper

TO THE QUESTION ABOUT MEAT FREEZING. REVIEW

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Key words: *meat, freezing, defrosting, quality, pathogens, fresh meat*

Abstract

The overview of studies of freezing and defrosting of raw meat, conducted during the recent years, is presented in the article. The freezing is the most effective method of preserving meat, so developments in this area are in demand by the food industry. There is noted the work on the creation of innovative technologies aimed at the optimizing of the freezing conditions (time, speed), reducing the loss of quality of the frozen products. Affected problems, which are appearing during the defrosting of meat, frozen in fresh and chilled condition. The interest of the use of the meat in the fresh state, which has not been in demand by the industry so far, is returning.

Introduction

The saving of the raw meat quality for a long time is possible due to refrigeration — freezing at low temperatures. Preventing changes in the properties of meat occurs by reducing the rate of microbiological, physico-chemical, biochemical and histological processes as a result of maintaining low temperatures.

In addition to long-term storage, freezing of meat solves many other problems: transportation to remote regions and countries of the world, regulation of supplies in accordance with processing, reducing the amount of food waste.

For such country with so large territory as Russia, the need of the usage of frozen meat will be relevant for a long time. Data on the volume of frozen raw meat in Russia [1] are presented in Table 1. From 9.2 to 18.0 % (not including 2014year, the record for the production of lamb) of meat produced in the country is subjected to refrigeration.

Frosted meat — steamed meat or chilled meat, subjected to refrigeration to a temperature in the muscle thickness at the depth 1 cm from minus 3 °C to minus 5 °C, at the depth of 6 cm — from 0 °C to 2 °C, during storage the temperature throughout the volume should be from minus 2 °C to minus 3 °C.

Fresh meat — meat, obtained immediately after slaughter and processing of carcasses or half-carcasses, having a temperature in the muscle thickness not lower than 35 °C. Cooled — obtained immediately after slaughter and processing of carcasses or half-carcasses, having a temperature in the muscle thickness not higher than 12 °C.

Chilled meat — steamed or cooled meat, cooled to a temperature in the muscle thickness from 0 °C to 4 °C. The shelf life is 16 days.

Frozen meat — fresh meat, or cold meat, or chilled meat, frozen to a temperature in the muscle column is not higher than minus 8 °C. Freezing of meat is carried out to preserve the quality and integrity of meat raw materials for its further use.

Deep freeze — the state of frozen meat, which has a temperature in the muscle column not higher than minus 18 °C. The beef with deep freeze can be stored up to 1 year, the pork — 6 months. This method of meat storage is the most reliable from the bacteria effects.

Meat dry (or shock) freezing — the meat, subjected to freezing in the air stream at the temperature minus 30–40 °C (i.e., almost instantly). The meat can be stored at moderate cooling (near 0 °C).

Defrosting meat — frozen meat, warmed to a temperature in the muscle thickness not less than minus 1 °C.

Main part

In the result of freezing the moisture, contained in the meat, came the ice as a result of heat removal at the temperature below cryoscopic. For example, the cryoscopic temperature for beef varies from minus 0.9 to minus 1.5 °C ($\Delta t = 0.6$ °C) depending on the pH level ($r = +0.73$, $P < 0.01$) [2].

In frozen meat in the form of crystals is the main mass water (more 70 %) [3]. Maximum crystal formation occurs in the temperature range from minus 2 °C to minus 8 °C [4].

Table 1. Dynamics of production frozen, frozen and thawed meat in Russia

Types of meat production (t.tons)	2010 year	2011 year	2012 year	2013 year	2014 year	2015 year
Frosted meat of the cattle, frozen, deep frozen and defrozen	43.0	38.6	36.0	1.6	43.2	51.7
Frosted pork, frozen, deep frozen and defrozen	57.6	61.6	58.5	67.5	97.1	108
Frosted lamb, frozen, deep frozen and defrozen, t	113	53.0	29.9	49.0	544	124
Total (% of total meat and byproducts production)	18.0	12.5	9.3	9.2	34.35	10.4

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Modern methods of freezing are aimed at achieving uniform crystal formation, the formation of small crystals, as well as preventing secondary crystallization, which leads to their increase. Large crystals are increasing the structural cell damage, which leads to the increase in the number of losses of the meat juice, the more intense loss of cell moisture and the high speed of the process of protein denaturation. These processes are reducing the quality of the defrosted product. However, large ice crystals are more stable in long-term storage than small ones. The process of increasing the size of ice crystals is faster at temperatures higher minus 18 °C, and is accelerated by temperature fluctuations during storage, transportation and realization [5].

The degree stage of the structure damage of muscle tissue also depends on the depth of autolytic processes in the meat to the time of freezing. The crystals are localized at the early stages of autolysis inside the muscle fiber during the meat freezing, violations of the structure are minimal. The greatest violations of the morphological structure occur when freezing meat in a state of rigor mortis. Optimal is the processing of meat in the early stages of autolysis with a high pH [4].

Additional difficulties are created by the size and thickness of the frozen cut or half-carcass, high parameters of which leading to uneven freezing and do not allow the one-time formation of crystals throughout the volume.

Depending on the type of freezing medium, freezing methods are divided into freezing in air, liquid coolants and cryogenic liquids (with liquid nitrogen or carbon dioxide). Depending on the speed of the process, which affects the formation of ice crystals, freezing is divided into: slow, intense and rapid. Rapid freezing contributes to the crystallization of water inside the cells, the formation of small crystals that reduce the degree of damage to the structure of cells.

Abroad, depending on the speed of freezing, the following methods are distinguished: cryogenic freezing > freezing in a high-speed freezer > freezing without air movement [6].

Innovation processes of freezing food are studying at the present time to optimize the settings of the freezing (rate and time) and product quality, using high pressure, using ultrasound, electromagnetic fields, mechanical vibration, etc. [7,8,9,10]. For this moment, none of these new methods have been widely used in the meat industry due to high capital and costs.

It is possible to get high-quality products through the use of cryogenic freezing technology, which allows you to freeze the meat for a few minutes using liquefied gas (liquid nitrogen temperature — minus 195 °C) or dry ice (temperature — minus 98 °C). The advantages of this method include the formation of microcrystalline structure, providing minimal loss of juice during defrosting, preservation of taste and presentation of the product, hygiene, reduction of losses from shrinkage, inhibition of aerobic microflora [6]. To reduce high costs and wide use of this method, it is necessary to minimize gas consumption per unit of production [11].

The possibility of using radiofrequency freezing in addition to cryogenic freezing is being studied. The pork, frozen with pulses of low voltage (2 kV) showed the best cell structure, less number of intercellular voids and less destruction of the cell structure. As a result of this treatment, small ice crystals are formed, well distributed in the intracellular region. The best microstructure of pork after radio frequency freezing is due to the ability of radio frequencies to reduce the freezing point [7].

With the use of high pressure (from 200 to 400 MPa), there are the following strategies for freezing food: freezing under high pressure, freezing under pressure, freezing with pressure relief [7].

Meat freezing technology with pressure relief («pressure-shift freezing»), provides for cooling the meat to a temperature of minus 20 °C at elevated pressure [6]. In case of sudden pressure relief in the muscle tissue, there is an instant microcrystallization, which leads to the formation of small uniform ice crystals. After defrosting the meat has improved quality due to minimal changes in tissue structure and reducing the degree of protein denaturation. However, studies on pork and beef have not shown commercial benefits of quality [10].

In order to study the effect of magnetic fields on the freezing process and the quality of meat raw materials (*M. longissimus dorsi from* black pigs) , a freezer was created, based on the principle of preserving the super-cooled state below the freezing point, due to the rotation of water molecules around its own axis, both inside and outside the cell under the action of magnetic fields — the «Cell Alive System «(CAS). The results of the study showed that freezing using magnetic fields (at minus 5 °C) resulted in a better moisture binding capacity compared to existing freezing methods (in an intense air flow at minus 40 °C, using immersion freezing at minus 65 °C) due to the size of ice crystals. They had small crystal sizes throughout the freezing time, and after defrosting, had no effect on the deterioration of meat quality [12]. The use of electro-magnetic vibrations during freezing allows to reduce the number of bacteria and store products for a long time (2–3 years).

In the works on the use of ultrasound, positive effects were obtained for both freezing and defrosting of products [13,14].

The use of microwave radiation during freezing significantly affects the crystallization process. The average size of ice crystals was much lower (62 %) than that of crystals formed during the traditional freezing process. The small size of the crystals resulted in less damage to the microstructure of meat [15].

The direction of research associated with the inhibition of recrystallization is developing. Secondary crystallization can be prevented by adding cryoprotectors (for example, proteins-antifreezes AFP [16], polyphosphates, sorbitol, sucrose, etc. or a combination thereof [6,17]. Their func-

tion is to reduce the temperature of the beginning of the crystallization of moisture, which leads to a change in the growth of ice crystals and slows recrystallization. Formed small ice crystals retain their size in the frozen product throughout the period of its storage.

Introduction of cryoprotectors into chilled pork as part of brine before freezing improves the quality of meat after defrosting (storage temperature minus 18 °C, storage time — 60 days.). In the experimental samples of the meat after defrosting, in contrast to the control samples, there was an increase in pH and water-binding capacity (reduction of meat stiffness), a decrease in damage to the structure of muscle fibers, the preservation of the structure of meat, the absence of undesirable sour smell [17].

Glycogen can be used as a cryoprotector [18]. With high hydration, glycogen can transfer into the bound state of intracellular water. Bound water does not undergo phase changes when exposed to low temperatures, reducing the amount of intracellular ice and crystal sizes. The high content of glycogen in meat can be provided by various feeding strategies, reducing stress in animals before slaughter and slowing the decomposition of glycogen in the process of autolysis.

Recently, interest in the use of fresh meat has been revived [19]. Unfortunately, the technology of meat processing in the steam state is not in demand by the industry, including the freezing of meat in the steam state.

In domestic enterprises of the meat industry, the freezing is mainly performed in the air in a single-phase or two-phase manner. Fresh meat is subjected to a single-phase method of freezing. The two-phase method provides for cooling of meat and its subsequent freezing in a cooled state [20].

The advantages of freezing meat in the steamy state are:
— reduction of processing time;
— less weight lose during the deboning (2–3 % compared to 8–10 % — in the cooled state);
— production of the meat with higher sanitary and hygienic characteristics;
— the possibility of using vertical deboning;
— reduction of refrigeration space and energy consumption.

The high initial temperature of meat is referred to the disadvantages (pork — 33–35 °C, beef — 36–38 °C), a short period during which its functional and technological properties are preserved (for pork -up to 3 hours, for beef, lamb and horse meat — up to 4–6) [19,21]. At the stage of defrosting of meat, frozen in the steam state, there is the effect of rigor-thaw-thaw-rigor (muscle rigor), due to the high content of ATP in the steamed meat. The combination of high pH (>6,4) and temperature < 12 °C results in a sharp reduction in muscle size [21], leading to a decrease in water binding capacity and structural and mechanical properties. To neutralize this effect, to preserve the quality and reduce high weight loss (6.8 times more than when defrosting beef in a cooled state) allows the use of pre-tempering of raw materials or its processing in a frozen state [22,23].

During the defrosting of the frozen meat to the cooled state, this effect is not observed. Meat subjected to two-phase freezing in chilled form, as a rule, has no organoleptic differences from meat frozen in steam form, but due to the formation of crystals of larger sizes, there is more damage to tissue structures and, accordingly, higher mass losses during defrosting [22].

Abroad, contrary to Russia, the meat industry is widely used freezing meat in the steam state.

Defrosting (*thawing*) or partial defrosting (*tempering*) of frozen meat is usually used at various intermediate stages of processing, as well as before the cooking [24] .

The quality of the defrosted product is influenced by the rate of enzymatic processes, as well as microflora, which has retained its viability during the freezing and storage of the product [25] .

One of the main problems of defrosting is the growth of microorganisms, therefore, when defrosting, temperature regimes should be strictly controlled [24].

Due to the processes occurring in the raw material as a result of storage, the original properties of meat after defrosting are not fully restored, even if the freezing and defrosting processes are carried out under optimal conditions. It is recommended that the defrosting process be carried out slowly, since in this case there is a more complete restoration of the structure of the meat and the binding of the protein structure of the defrosted water, which reduces the loss of meat weight [26] .

As a result of temperature decrease in the process of freezing in meat raw materials the course of microbiological and biochemical processes changes. When storing meat raw materials and meat products below minus 10 °C, the growth of microorganisms and enzymatic processes are practically stopped, which minimizes quality losses, but does not stop until the end [6].

The microorganisms, depending on the ability to survive after freezing, frozen storage and defrosting, can be divided into:

- resistant (including *Listeria, Staphylococcus* and *Streptococcus spp*.; survive up to 50 % of gram-positive bacteria),
- moderately resistant (most gram-positive bacteria, fungi, and some yeast species) ;
- sensitive (gram-negative bacteria), freezing and storage in a frozen state does not guarantee complete removal of these «sensitive» organisms from frozen foods) [27].

The minimum temperature of growth of microorganisms depends on factors such as pH, water activity (salt concentration) and the presence of oxygen.

It was experimentally established that the beginning of intensive reproduction of microorganisms on the surface of meat during defrosting is in the low plus range — at a meat temperature of 7 °C [28].

As a result of freezing, many pathogenic microorganisms die, others return to life under favorable conditions. The mechanism of damage to microbial cells during freez-

ing occurs as a result of mechanical defects caused by ice crystals, drying due to reduced water activity and oxidative damage [10]. Further research is needed to understand the physical and chemical interactions of the food matrix with the microbial cell during freezing and low-temperature storage, and the conditions under which the latter is able to restore its viability. This will identify ways to prevent the recovery of microbial activity. The understanding of these processes will lead to the fact that freezing in the future can be used as a reliable way of preserving food from food pathogens [29].

The safety of thawed meat can be guaranteed provided that the feedstock is of high quality and the refrigeration chain is stable.

In studies on the dynamics of the quantitative content and total composition of pathogenic microflora during storage of frozen beef with different initial microbial state, it was found that when storing beef in a frozen state, the rate of development of psychrotrophic microflora is 3.0–32.6 times higher than mesophilic. It is also noted that the higher the initial microbial contamination of beef, the faster the rate of development of microflora in the process of storing meat in a frozen state, which helps to reduce the shelf life of frozen beef and reduce the hygienic and technological quality of meat. Therefore, when choosing the conditions of meat storage (temperature, duration), the initial microbial state of the carcass surface is important — the amount of psychrotrophic microflora [30].

In the process of freezing and storage, hygienic problems may arise, leading to the detection of pathogenic microflora in frozen meat. Therefore, currently there is a need on the part of the meat industry for simple and fast methods of detection of pathogenic microflora in frozen meat, including *Salmonella*, thermophilic *Campylobacter spp*. and other pathogens [31,32,33].

All these factors must be taken into account when defrosting food products.

The defrosting process is undesirable to be carried out slowly, at temperatures above 10...15 °C , as conditions are created for the rapid growth and reproduction of pathogenic and harmful organisms [27].

Processes that reduce the quality of meat are accelerated at storage temperatures, especially above minus 5 °C (minus 5... minus 15 °C). For example, oxidation of myoglobin (in fat deposits, in intramuscular lipids of lean meat due to oxidation of phospholipids) occurs most rapidly at a temperature of minus 10 °C.

Inhibition of growth of pathogenic and putrefactive microflora, it is possible to achieve with bioconservative with the use of protective cultures (e.g. lactic acid bacteria) survive in the cold processing, physical processing methods (ionizing irradiation, pressure treatment, etc.), packaging (including packaging under vacuum and in a gas atmosphere, active packaging), comprehensive effects (including a reasonable label) [34].

After defrosting, meat should either be processed immediately or stored at temperatures below 4...5 °C or subjected to culinary treatment. The growth rate of microorganisms in thawed meat will be the same as in chilled meat (not frozen) under the same conditions of cold storage [24]. Defrosted at different periods of cold storage, during the next 2 days after defrosting at a temperature of 0 °C to 4°C retains the stability of microbiological parameters, normalized SanPiN 2.3.2.1078 and in sanitary and hygienic terms is completely safe for the production of meat products (data of the V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences).

Conclusion

Freezing, as a method of preserving food products, will remain relevant in the near future, as solving of many problems associated with the processing of meat. Research on the development of innovative freezing technologies are carried out in the direction of ensuring minimum losses of quality and safety of food products. The main problem of their widespread use remains high capital and operating costs. Cost reduction at Russian enterprises can contribute to the freezing of meat in the pair condition. Despite the numerous studies carried out on the freezing of fresh meat, and the advantages of this technology, so far the use of meat frozen in the steam state has not been widely used in domestic enterprises of the meat industry, which necessitates further research in this direction.

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