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THEORY AND PRACTICE OF MEAT PROCESSING

ТЕОРИЯ И ПРАКТИКА ПЕРЕРАБОТКИ МЯСА

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Original scientific paper

ASSESSING THE EFFECT OF THERMAL TREATMENT ON MEAT PROTEINS USING PROTEOMIC METHODS

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Key words: *muscle proteins, stuffing, meat, two-dimensional electrophoresis, thermal treatment*

Abstract
The results of studying the effect of various temperatures on the protein composition of minced meat from porcine m. longissimus dorsi by two-dimensional electrophoresis are presented. The most complete distribution of protein fractions was observed in fresh raw minced meat, and when it was exposed to negative temperature, there was a sharp decrease in protein components (carbonic anhydrase 3, αβ-crystallin), as well as a decrease in the staining intensity of protein spots of the main constitutive fractions (tropomyosin alpha 1, myosin light chain 1). In the case of heat treatment, structural muscle proteins were retained with some changes in high molecular weight fractions, namely, protein molecules degraded to compounds with a simpler structure. It was noted that fractions of tropomyosin β-chain, triosephosphate isomerase 1, myosin light chains 2 were not detected after minced meat was frozen, while tropomyosin alpha 1 was retained in all samples.

Introduction

It is known that introduction of processing technologies with different temperature variations is of crucial significance to meat technological characteristics [1,2]. The conditions of treatment, especially temperature, cause a cascade of chemical and physical changes that affect meat proteins. Occurring protein modifications include denaturation and aggregation [3,4], fiber shrinkage and collagen solubilization [5], the Maillard reaction [6] or protein oxidation [7]. Therefore, a range of used temperature conditions for meat processing and its following preparation leads to potentially different impacts on protein modification.

If meat products are exposed to the reactive oxygen species, the oxidative stress is induced by proteins, which cause chemical destruction, changes in protein functionality, loss of essential amino acids and possibly product digestibility [8]. With occurrence of oxidation, not only basic and aromatic amino acids are transformed into carbonyl, but also thiol groups are replaced with formation of disulfide bridges, which cause polymerization and subsequent aggregation of proteins [9]. Oxidation of aromatic amino acids can lead to denaturation, polymerization, aggregation, fragmentation, changes in hydrophobicity, solubility, gel formation and emulsification, which ultimately affect the physico-chemical condition of proteins [10].

Therefore, the main aim of the research was to understand how an impact of different temperatures (freezing and heat treatment) can cause biochemical changes in meat systems, which can affect food quality of meat proteins, using comparative analysis of two-dimensional maps.

Materials and methods

The object of the research was minced meat from porcine m. longissimus dorsi: raw, raw after freezing at a temperature of minus 40 °C and heat treatment, namely cooking until reaching 68 °C in the sample center. Minced meat was homogenized using a cutting mill Retsch GM 200 (Retsch GmbH, Germany) with a gradual increase of the knife revolution speed from 2000 rpm to 5000 rpm.

Two-dimensional electrophoresis (2-DE)

The samples described above were studied by the method of two-dimensional electrophoresis (2-DE). At the first stage, isoelectric focusing (IEF) was performed at 3650 V/h in tube gels (2.4 mm × 160 mm); 0.02 M orthophosphoric acid and 0.02 M sodium hydroxide were used as the anode and cathode buffers, respectively. After IEF, gels were incubated for 10 min. in 2.5 ml of the equilibration buffer I (6 M urea, 20% glycerol, 2% SDS and 1% DTT in 50 mM tris-HCl buffer, pH 8.8), then in equilibration buffer II (6 M urea, 20% glycerol, 2% SDS and 4% iodoacetamide in 375 mM tris-HCl buffer, pH 8.8).

After that, electrophoresis with sodium dodecyl sulfate was carried out. For this, the equilibrated gels were transferred to the 12.5% polyacrylamide gel (170 mm × 180 mm × 1.5 mm). Electrophoresis was performed using the buffer contained 25 mM tris -HCl, 192 mM glycine and 0.1% SDS at 30 mA on the gel until the dye front reached the end of the gel [11,12].

Visualization of the protein fractions and image analysis

Protein spots were visualized by staining with Coomassie Brilliant Blue G-250. Two-dimensional electrophoregram in the wet state were used for computer densitometry. Their digital images were obtained using a Bio-5000 plus scanner (Serva, Germany). Scanned images were analyzed with the software package ImageMaster ™ 2D Platinum based on Melanie 8.0 (GE Healthcare and Genebio, Switzerland), which automatically detected and quantified protein fractions. Then digitized 2 DE images were compared by the matching method.

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Results and discussion

Two-dimensional (2D) electrophoregram of investigated minced meat presented in Figure 1 showed similar distribution of fractions that corresponded to the multi-module database «Proteomics of Muscular Organs» [13] regarding *Sus scrofa*. The greatest quantity of protein fractions was found in native/raw minced meat, while a noticeable decrease in protein quantity was observed in the sample after freezing. Decomposition of the group of protein fractions with a molecular weight range of 65 kDa to 250 kDa was revealed in minced meat after heat treatment (Figure 2). It is interesting that tropomyosin beta chain (TPM2 33.5 kDa; pI 4.71), was not detected after freezing; with that, the fraction of tropomyosin alpha 1 (TPM1 33.5 kDa) was retained in all samples.

In addition, the degenerative effect on the myosin light chains 2 (MLC2 18.5 kDa; pI 4.89) was revealed both at the negative and positive temperatures. The similar picture was found for the fraction triosephosphate isomerase 1 (TPI 1 23.0 kDa; pI 6.80), which was intensively pronounced in raw minced meat, absent in the sample after freezing and found in small amounts in cooked minced meat. A uniform decrease in the staining intensity of protein spots of carbonic anhydrase 3 (30.5 kDa; pI 7.55), αβ-crystallin (20.0 kDa; pI 7.60) and myosin light chain 1 (MLC1 21.0 kDa; pI 4.90) from native minced meat to frozen was observed.

Conclusion

Various thermal treatments of meat systems differently affect the protein profile, in particular, the quantity of proteins and their staining intensity. Using the proteomic analysis by the method of two-dimensional electrophoresis, the greatest quantity of fractions with the pronounced intensity of the protein spots was found in native raw minced meat.

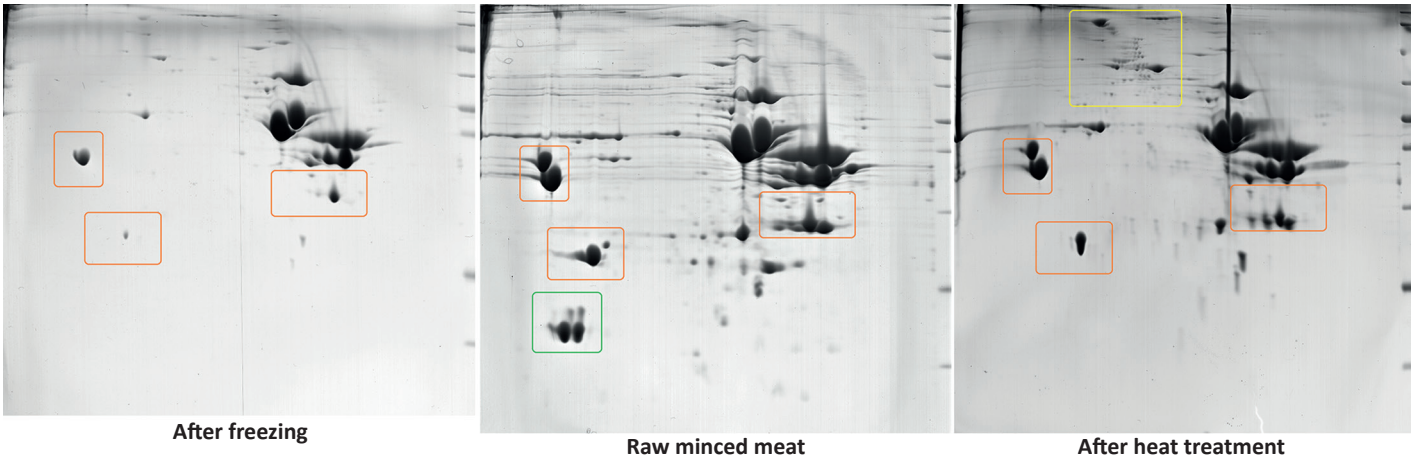

Figure 1. 2D electrophoregram of minced meat with different thermal treatment

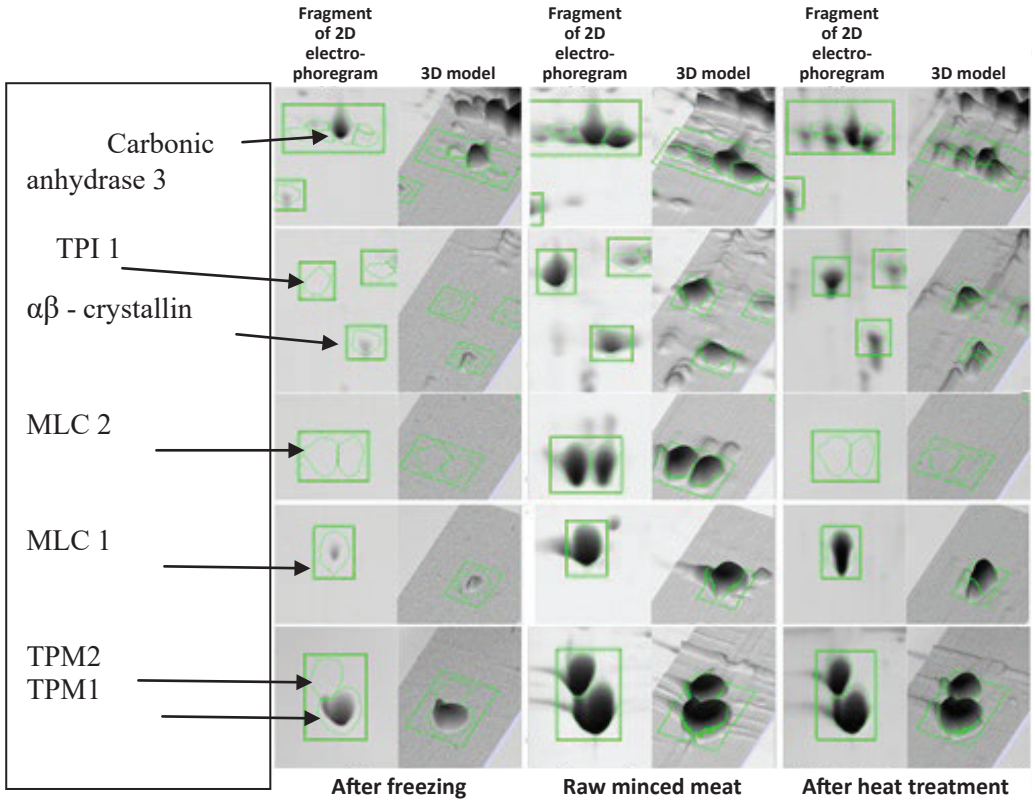

Figure 2. Enlarged fragments of 2D electrophoregram

In the case of sample freezing, part of protein compounds (triosephosphate isomerase 1, myosin light chains 2 and tropomyosin beta chain) was lost, which was caused by the mechanical impact of water crystals on the muscle fiber walls and protein denaturation due to water separation from the protein substance. It was found that in the sample after heat treatment, several proteins with the molecular weight higher than 65 kDa disintegrated into a set of spots of fractions of the same name that were present in the native minced meat, and part of proteins was not detected on the 2D electrophoregram.

These interpretations can ensure understanding of an effect of different food processing strategies and focus attention on biofunctional and nutritional properties of meat proteins.

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Original scientific paper

SUBSTANTIATION OF THE METHOD OF MEAT SAMPLE PREPARATION FOR INSTRUMENTAL DETERMINATION OF CONSISTENCY

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Key words: *consistency, pork, beef, sample preparation, shear stress*

Abstract

In recent years, there has been a transformation in the choice and lifestyle of Russian citizens. An increasing part of the population makes a more reasonable choice, taking into account all aspects, including the most important for meat products — these are organoleptic characteristics, in particular the consistency of the product. Consumers prefer products with the delicate, soft texture, expect good «biteness» and «cheweness». Therefore, preference is given to tender, juicy meat with a low content of connective tissue. The most commonly used method for testing meat consistency in laboratories around the world is a method that uses strength testing machines with a Warner-Bratzler blade (WB blade). In this work, the Shimadzu AGS-1kN universal testing machine (Japan) was chosen for research. Samples obtained from l. dorsi of pork and beef were selected as meat raw materials. To determine the optimal and reproducible method of sample preparation, some of them were subjected to heat treatment before analysis. In the study of samples without heat treatment, deviations from the average were more than 11%. After meat was cooked, a decrease in the relative standard deviation of the maximum shear stress from the average was achieved: from 11% in raw meat to 5% in a pork sample and 5.3% in a beef sample. The heat treatment of pre-cut samples led to a change in their geometric shape, which created additional difficulties for obtaining correct results, and also negatively affected the increase in the relative deviation to 15.5% for beef.

Introduction

Despite an emergent decline in meat product output over the first six months of 2019, producers should make efforts to retain their consumers. In recent years, a choice and lifestyle of the Russian citizens have been transformed. An increasing proportion of the population makes a more reasonable choice that takes into consideration all aspects, including those that are the most important for meat products — organoleptic characteristics, in particular, product consistency. This is confirmed by the results of investigations performed by foreign scientists who demonstrated that one of the main meat quality indicators noted by consumers was tender [1]

Based on consumer preferences, producers and developers of meat products have begun to pay more attention to formation of tender consistency of a finished product. At the same time, it was found that methodological approaches to objective assessment of meat and meat product consistency as well instrumental product characteristics in existing regulatory documents were absent. Analysis of scientific-technical literature allowed revealing several criteria for assessment of meat product consistency. Ball et al. made attempts to present meat consistency as a complex of two sensations: visual — «sight» and tactile — «feel». They meant by the term «sight» that meat consistency is the macroscopic peculiarities of muscle tissue in terms of smoothness or fineness of grain. It was suggested that consistency depends on a size of fiber bundles: the smaller bundles, the better consistency. On this basis, this assessment was applicable both to raw and cooked samples. On the contrary, the term «feel» could be applied only to ready-to-eat samples as product consistency was regarded as the feel of smoothness or fineness of muscle tissue in the mouth during product chewing [2].

Later on, it was found that assessment of consistency only by a size of muscle fibers and/or bundles of muscle fibers seen on the cross section of the surface was not objective. Meat consistency depends on a complex of different characteristics and factors. Both lifetime aspects (such as age, breed, gender and diet of animals, type of muscles and anatomic location) and several technological factors (for example, the use of electrical stimulation, a method of processing) affect consistency [3]. Consumers prefer products with tender, soft consistency, and expect good «biteness» and «cheweness» [4]. Therefore, preference is given to tender and juicy meat with an insignificant content of connective tissue [5].

The most common method for investigation of meat consistency in laboratories of the whole world is a method that uses the strength testing machines with the Warner-Bratzler blade (WB-blade). The principle of this method is based on modeling of food product chewing [6]. It was developed by Lyman Bratzler in 1932, and already since the 1950s, this method has been used worldwide. Due to good reproducibility of results, this method can be used for detection of hardness and tenderness of meat products. It is also intended for detailed analysis, for example, for studying dependencies associated with used methods of selection and their effect on the ultimate meat quality [7,8]. At the same time, an absence of the standardized methodological

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approach does not allow obtaining comparable results in different laboratories. It is the process of sample preparation that is of great importance in this case.

Materials and methods

The universal testing machine Shimadzu AGS-1kN (Japan) was chosen for experiments. The method is based on WB-blade passing across the fibers of a sample, which has a shape of a parallelepiped with a length of 50 mm, width and height of 20 mm. The blade speed was 50 mm/min. The force was registered with a strain gauge. The obtained results were presented in TrapeziumLite X software and subjected to statistical processing.

The samples of l. dorsi from pork and beef were chosen as meat raw materials. To determine an optimal and reproducible method of sample preparation, some of the samples were subjected to heat treatment before analysis. Heat treatment was carried out by cooking in a water bath at a constant temperature of 80 °C until reaching a core temperature of 72 ± 1 °C by two methods:

1. using a whole piece with following cooling and excision of a sample, which had a shape of parallelepiped with a length of 50 mm, width and height of 20 mm.
2. cooking a preliminary excized sample, which had a shape of parallelepiped with a length of 50 mm, width and height of 20 mm.

Meat consistency was assessed by a value of maximum shear stress, which accounts for the geometrical shape of samples and Warner-Bratzler blade width (equation 1).

$$Q = F/S \times k, \quad (1)$$

where:

Q — maximum shear stress, N/m^2,

F — maximum shear force, N,

k = 0,0015 — coefficient accounting for Warner-Bratzler blade width.

Results and discussion

At the first stage, maximum shear stress of the control samples, which were not subjected to heat treatment, was determined. The obtained results are presented in Figure 1 and Figure 2 and in Table 1, respectively.

Table 1. Mean value of maximum shear stress of pork (C) and beef (Cb) samples without heat treatment

Sample	Mean value of shear stress, Pa
Pork (C)	101.2 ± 11.3
Pork (C)	105.1 ± 11.5

The results suggest that the obtained values for both samples are in quite a close range. However, in both cases the observed measurement results indicated a rather large deviation between samples: from 120.9 to 84.1 Pa for pork and from 130.6 to 84.0 Pa for beef. The obtained deviations can be explained by the morphological structure of meat raw materials, its heterogeneity, the presence of connective and fatty tissues besides muscle fibers. Connective tissue has high strength and when it comes to the cutting edge of the Warner-Bratzler blade during analysis, it shows much higher

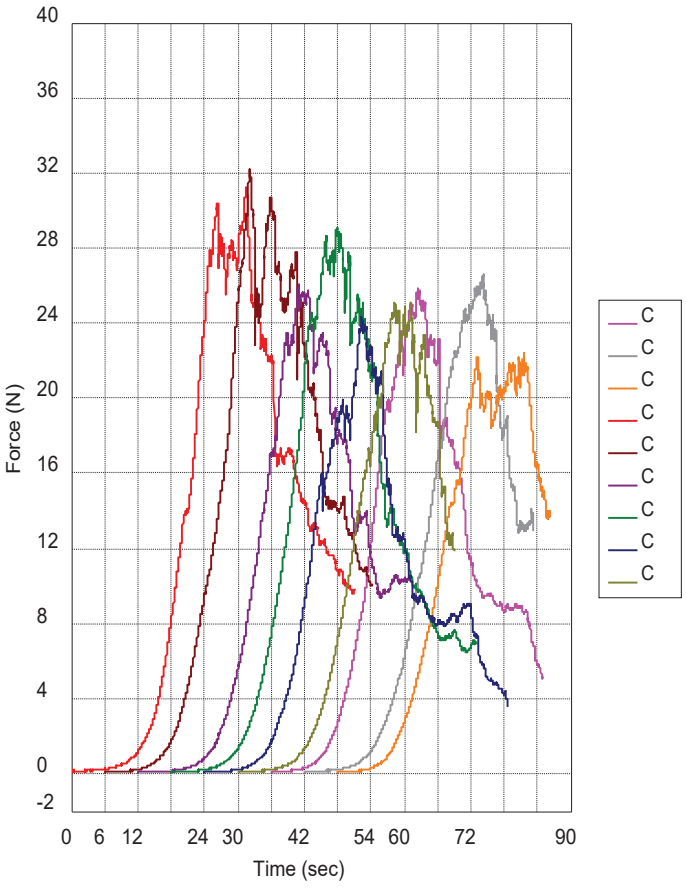

Figure 1. Dynamics of the shear force change during pork sample cutting

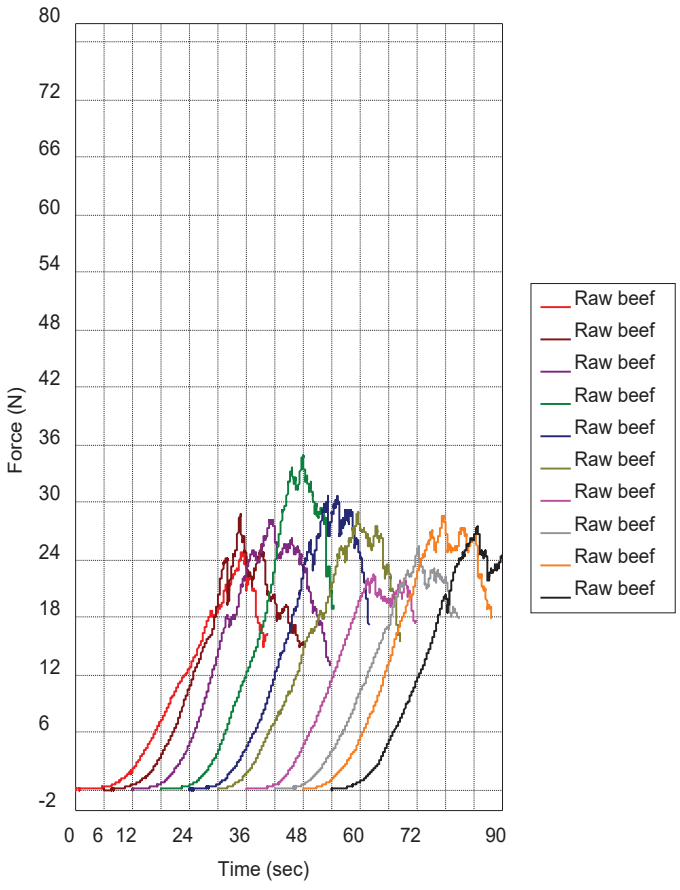

Figure 2. Dynamics of the shear force change during beef sample cutting

resistance to cutting than muscle tissue. In the analysis of the uncooked samples, therefore, deviations from the mean value can be more than 11% provided that one of the most homogeneous muscle in the cut (*m. longissimus*) was chosen for an experiment. This large deviation does not allow making a statement about objectivity of a method and reproducible results. At the same time, virtually identical results for uncooked samples from different meat raw materials (pork and beef) does not enable meat differentiation by type of farm animals using this method, while it is acknowledged that cooked beef is tougher than pork.

Taking into consideration the obtained results, the strength properties of meat raw materials after cooking were studied. Cooking was chosen as the most common method of heat treatment. To determine a method of sample preparation before or after heat treatment, two different sequences of steps were used: heat treatment followed by sample excision or, vice versa, a sample was excised from a whole piece and then cooked.

The results of ten parallel measurements of maximum shear stress for cooked pork samples are presented in Figure 3 (sample 1.1 — the pork sample excised from the cooked whole piece, sample 1.2 — the pork sample excised from the raw whole piece and then cooked).

It is worth noting that the mean of maximum shear stress in the sample cooked in the cut form (1.2) was almost twice as high as the mean in sample 1.1: 114.4 Pa for sample 1.1 and 239.2 Pa for sample 1.2, respectively. It can be explained by the structure and technological characteristics of meat raw materials. However, most significant is the fact that a reduction in the relative standard deviation of the values of maximum shear stress from the mean was achieved: from 11% in raw meat to 5% in sample 1.1 and 8.2% in sample 1.2.

The similar picture was observed for the studied beef samples (Figure 4). Beef sample 3.1 was prepared in much the same way as sample 1.1, sample 3.2 as sample 1.2.

It is worth noting that in both cases the beef samples became tougher: the mean value of maximum shear stress increased from 105.1 Pa in raw meat to 198.1 Pa in sample 3.1 and to 239.5 Pa in sample 3.2. This result was expected as beef toughness always strongly increases after heat treatment. The relative value of deviation in the case of sample 3.1 was 5.3%, which was very close to the result in pork. In the case of sample 3.2, the relative deviation increased from 11% to 15.5%.

Therefore, the following conclusions can be made.

When a sample was excised from a cooked piece, the value of maximum shear stress after pork heat treatment insignificantly exceeded the similar value in raw meat — 114.4 Pa and 101.2 Pa, respectively. However, heat treatment allowed reducing the value of relative deviation in parallel sections from 11 to 5%, increasing reproducibility of results; preliminary excision of samples from raw meat and their following cooking increased the value of maximum shear stress almost twice up to 239.2 Pa upon an insignificant decrease in relative deviation up to 8.2%.

The differences were even more significant in beef: after cooking and following excision of a sample, the results of maximum shear stress were twice as high as the similar value in raw meat: 198.1 and 105.1 Pa. Moreover, the value of relative deviation decreased from 11 to 5.3% similar to what was observed in pork.

Conclusions

According to the obtained data, it is not recommended to analyze texture of meat samples by the method of maximum shear stress measurement using the Warner-Bratzler blade on meat raw material samples without preliminary heat treatment. The values of shear stress in raw meat samples had higher standard deviations (more than 11%). In addition, determination of the shear stress value in raw meat does not allow muscle tissue differentiation by animal species.

For correct sample preparation, it is recommended to apply initial heat treatment of a whole meat piece by cooking

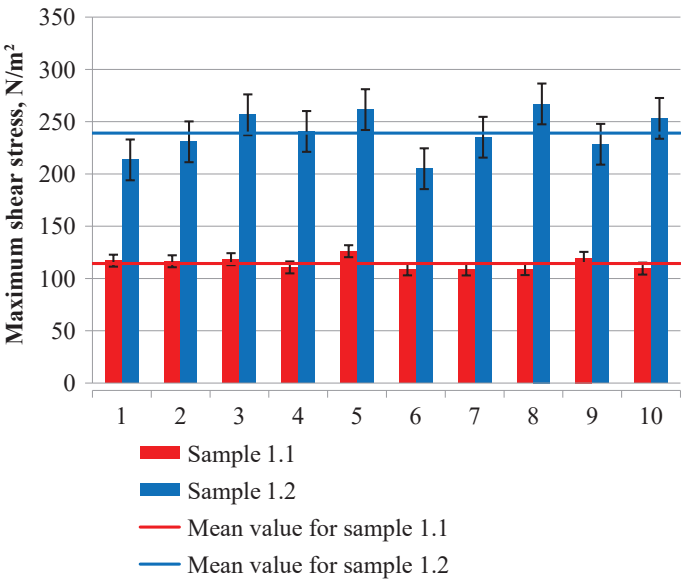

Figure 3. Results of maximum shear stress measurement in cooked pork samples

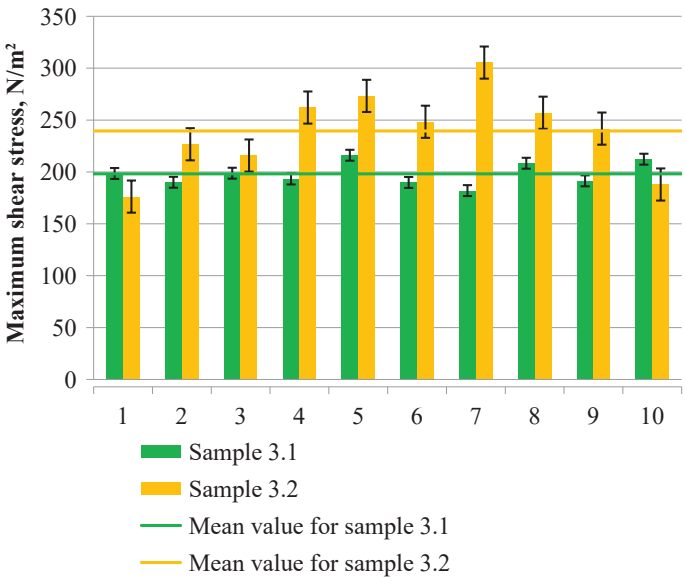

Figure 4. Results of maximum shear stress measurement in cooked beef samples

in a vacuum package up to a core temperature of 72±1 °C, and then to excise a sample of a necessary geometric shape. This preparation of samples will allow obtaining the lowest values of relative deviations both for pork and beef: 5 and 5.3%, respectively.

Heat treatment of preliminary excised samples led to changes in their geometric shape, which created additional difficulties for obtaining correct results, and also negatively affected an increase in the value of relative deviation to 15.5% for beef.

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Original scientific paper

DEVELOPMENT OF A PERSONALIZED MEAT PRODUCT USING STRUCTURAL-PARAMETRIC MODELING

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Key words: *individual nutrition, personalized diets and products, digital nutritiology, meat product*

Abstract

At present, there is no consistent definition of the term «personalized nutrition». The paper presents existing descriptors in this field of food science: precision nutrition, nutrigenomics, nutrigenetics, individual nutrition and so on. It is noted that cardiovascular diseases occupy the first place among noninfectious diseases associated with malnutrition. Optimal nutrition leads to a reduction in the risk of their occurrence. The methodology of structural-parametric modeling, which allows designing personalized optimal human nutrition based on medical indicators, is presented in terms of minimization of the risk function. The algorithm of a substantiated optimal choice of mass fractions of components (ingredients) of the food recipe composition is given. The main descriptors of a food product with the antisclerotic action for its designing using structural-parametric modeling are shown

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Introduction

One of the main prerequisites for maintaining human health is an optimal diet containing necessary macro- and micronutrients.

The results of the studies from 1990 to 2016 (Figure 1) show that the countries of the former USSR occupy 14 of the 20 top places in the rating of deaths from diseases associated with unhealthy diet [1].

According to the research data, cardiovascular diseases are first among noninfectious diseases associated with malnutrition. One of the main prerequisites for maintaining human health is an adequate diet [2]. With the optimal structure of nutrition, high working capacity and primary prophylaxis of many diseases are ensured, immune resistance is increased and protection of the body against exposure to unfavorable environmental factors is enhanced.

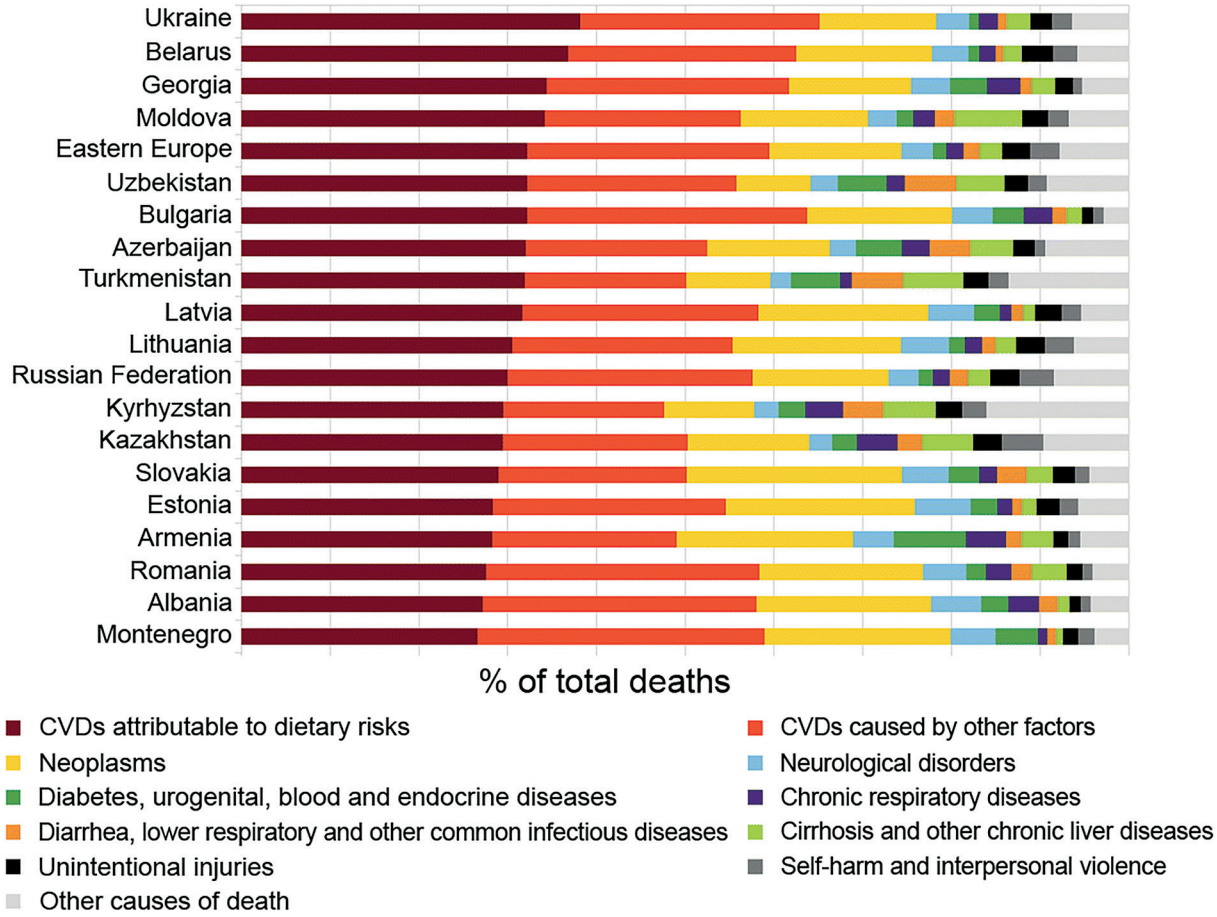

Figure 1. Rating of countries by deaths and their reasons [1]

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Production of healthy food was organized for the first time in Japan in 1955. Regulation (EC) No 1924/2006 «On nutrition and health claims made on foods» [3] gives definitions of the terms «nutrition claim», «reduction of disease risk claim», «health claim».

The concept «Society 5.0», which offers the deeper and extended use of digital technologies in all spheres of human life, was presented in Japan in 2016 (Figure 2). According to the strategy «Society 5.0», the advanced technologies entering all life spheres should lead to appearance of new business forms and types and, thereby, to economic rise of the country in general and a growth in life quality of every person individually.

As Figure 2 shows, one of the sustainable development goals of Society 5.0 is creation of «smart» functional foods for personalized human nutrition.

Academician Tutelyan V. A. [5] noted that integration of nutritiology with engineer sciences has been increasing day by day, particularly in the case of food technologies, which creates opportunities for the development of new progressive methods and techniques for designing products with the targeted chemical composition, specialized products as well as diet personalization. The future of integrative nutritiology is creation of digital nutritiology.

The problems of individual (personalized) adequate nutrition of a particular individual with consideration for multiple parameters of the state, alternatives and criteria, different constraints and conditions can be solved using computer technologies of processing and formalization of knowledge with finding optimal solutions based on models and methods of multi-criteria structural-parametric optimization and objective assessment of adequacy of proposed options.

The paper presents the methodology for the development of structural-parametric models of personalized adequate nutrition, formalization of a knowledge base and creation of an expert system for analysis and correction of a daily diet and nutrition regime for determined human groups with regard for metabolism of nutrients (ethnicity, cultural preferences, health status, lifestyle and clinical factors) from available traditional products in a certain region and modeling an individual (personalized) product by the example of a meat product with antisclerotic action.

Materials and methods

The conceptual approaches of computer design of foods with targeted quality characteristics are based on the principles of structural parametric modeling and optimization of a nutrition system, choice of different types of raw materials and ratios of recipe ingredients, which in combination allow obtaining a composition that corresponds to the fullest extent to the medico-biological requirements and indicators of the nutritional and biological value in terms of the quantitative content and quality composition of nutrients.

Figure 2. Society 5.0 for sustainable development goals [4]

The structural-parametric model [6,7] of a multi-component product reflects functional links between the parameters of the composition and properties of desirable products, as well as many specific factors and ratios that determine the goals, purpose and the use of products under development intended for particular consumer groups.

An advantage of the structural-parametric model of a multi-component product resides in the fact that it permits accounting for the whole variety of factors of nutrient influence on the processes occurring in the human body depending on its metabolism and current physiological state. It provides the means of determining regularities, which knowledge will make it possible to quite confidently talk about possible deviations in a diet and reasons of their appearance, and to find a solution to correct misbalance in the essential elements.

Results and discussion

Personalized nutrition pursues the idea of individualization [8]; recommendations and advice on nutrition should not be built upon average statistical norms for consumption of nutrients applied for age/gender groups of population differentiated by a level of physical activity.

In the personalized nutrition like in other scientific fields, multiple concepts and descriptors are used at the early stage of the development, for example, precision nutrition, stratified nutrition, tailored nutrition.

- Stratified and tailored nutrition are similar. These approaches consist in grouping people with common characteristics and giving recommendations that are suited to each group.
- Personalized nutrition and individually tailored nutrition signify similar concepts and make the following step forward in an attempt to provide nutritional intervention/recommendations that are suitable for a certain person. According to the concept of individually tailored nutrition, many factors are taken into account (such as age, gender, physical activity, anthropometrics, ethnicity, cultural preferences, health status, lifestyle and clinical factors) when developing a diet/food product; however, the parameter of epigenetics is not considered.
- Precision nutrition is the most «ambitious» of the descriptors. It suggests that it is possible to have sufficient quantitative understanding of the complex relations between a phenotype of a person (including health) and his/her food consumption. Based on these relations, recommendations on an individual diet are built. For precision nutrition, a lot of knowledge and regularities with a high degree of scientific confidence are required.

With transition from stratified to personalized and precision nutrition, it becomes necessary to use more and more parameters or characteristics for achieving the targeted goal. For example, stratification can be accomplished using one or several parameters, such as age, gender or health status. In personalized nutrition, it is necessary to account for the complexity of relationships between an individual diet and phenotype; to achieve the goal of precision nutrition, it would be necessary to use a wide range of parameters, possibly, including «big data» approaches.

At this stage of the research, the authors adhere to the concept of designing diets/products of «individually tailored nutrition».

It is necessary to pay attention to two aspects:

1) physiological reactions on products/nutrients;
2) individual behavior models, including preferences, barriers and motives.

Computer expert systems can take into consideration many personal data, such as eating habits (including personal preferences), health state, physical activity, characteristics of sleep and so on. Data analysis and combination can increase the value of health and well-being management for individuals.

Table 1 lists a range of constant to changeable factors influencing human health [9].

Some of the factors (such as smoking, diet and physical activity) can be changed throughout the life, while age or heredity is unchangeable.

Factors influencing health can increase (for example, a high calorie diet combined with low physical activity or its complete absence) or reduce (for example, eating food rich in fibers) a risk level for an individual.

Clinical trials of these factors (biomarkers, such as serum glucose level, blood pressure and so on) show an effect of a diet/food product on health.

When developing an optimal individual food product, the authors propose a dialog algorithm (Figure 3) [10] for determination of the component composition (raw materials of animal and plant origin), their quantity and ratio by specified criteria and constraints. The first stage begins with entering information about an existing daily diet of patients with consideration for their taste characteristics, ethnic traditions, region of living and so on.

Table 1. Factors influencing health

Unchangeable	◄———	———	Easily changeable
• Age	• Medical services	• Education	• Diet/ eating habits
• Gender	• Social structure	• Time for preparation	• Physical activity
• Ethnicity	• Political conditions	• Culinary skills	• Sleep
• Genetics	• Environmental pollution		• Stress
• Family history	• Values		• Hygiene
• Culture	• Purchasing power		• Pharmaceutical drugs (narcotics)

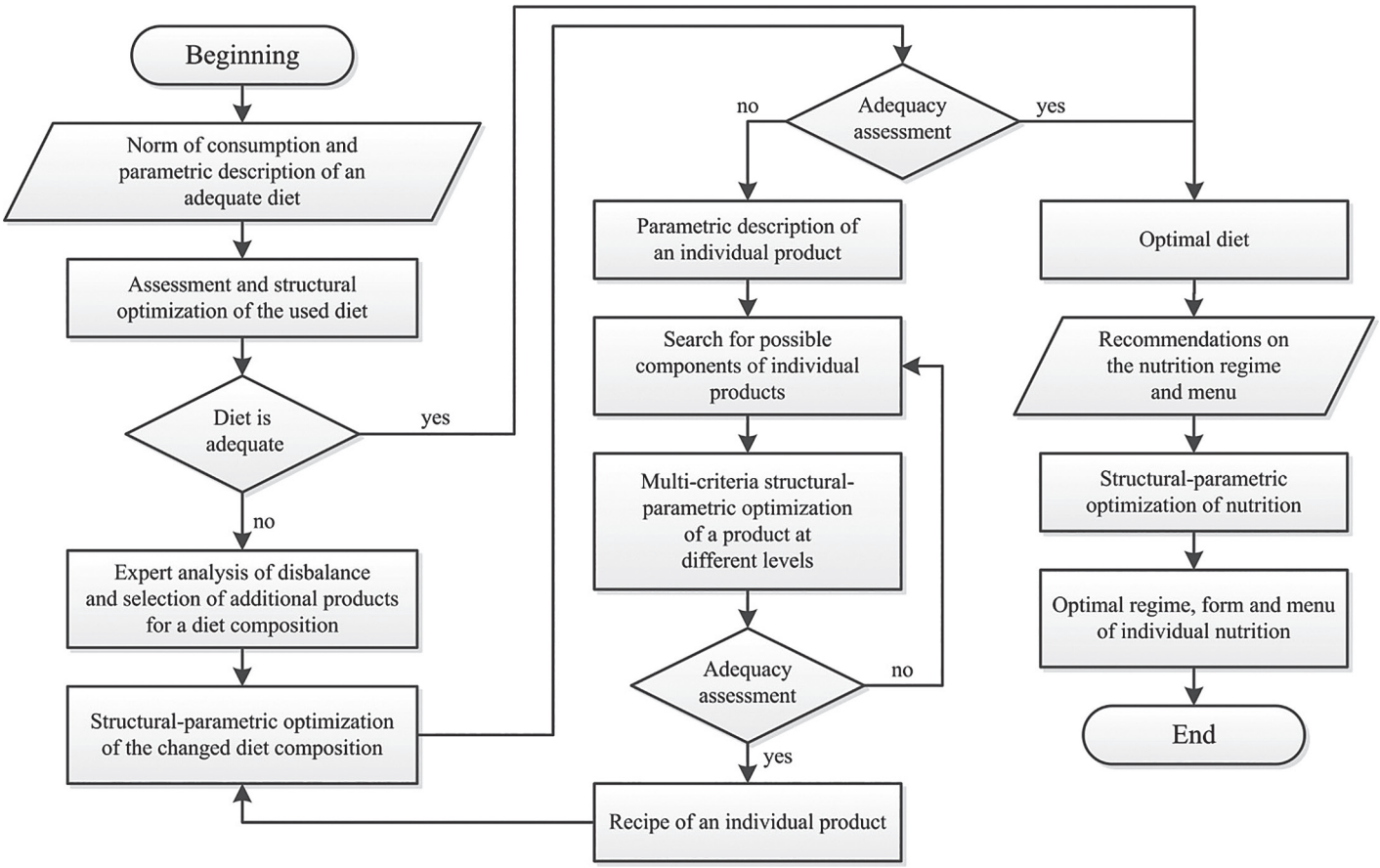

Figure 3. Dialog algorithm of the structural-parametric optimization of the adequate nutrition [10]

Using the described medico-biological status of a person, a parametric model of his/her adequate nutrition is formed in the expert system in terms of specific parameters, norms and ratios of the nutrients and components that are required daily. On this basis, assessment of an existing daily diet is carried out with the normative parametric structure of the indicators of adequate nutrition.

The hierarchy of the quadratic criteria [11] of the minimal deviation from the reference structure of the set of indicators for nutritional, biological and/or energy values, as well as the criteria of protein digestibility, adequacy of protein intake, deficiency of albumin, transferrin, lymphocytes and others are used as a targeted function.

Minimization of the possible noncoincidence between parameters of the «standard» and proposed diets is linked with multi-criteria optimization and formation of the Pareto-optimal set of solutions by formalized criteria.

Upon insufficient compensation of deviations by selection of desired products and dishes that are constituents of a diet, an individual combined functional or specialized product that minimizes established deviations is modeled.

The main task of the development of the individual functional or specialized food product is replenishment of deficient substances in a diet bringing their content to the norms that correspond to the metabolism of nutrients in the human body.

By the example of designing a recipe and product technology for gerodietetic nutrition, let us examine the methodology of structural-parametric modeling of a specialized, functional product.

To this end, the parametric description of scientifically substantiated principles of «diet therapy» for the elderly is downloaded from the Information bank that contains information and knowledge about nutrition of determined groups of people with different noninfectious diseases.

The main input parameters, as was noticed above, are *age* (in our case more *than 60 years*), gender (males), weight (excessive body weight, body mass index more than 30 (BMI ≥ 30), arterial pressure (elevated blood pressure); as an additional factor, an atherogenicity index (an elevated atherogenicity index) is taken into consideration.

When designing nutrition for the elderly, it is necessary to take into consideration the following:

— energy balance of nutrition with regard to actual energy expenditure of the body;
— prophylactic orientation of nutrition and not only regarding *atherosclerosis,* but also other common pathologies of the old age (obesity, diabetes mellitus, hypertonic disease, oncological diseases, osteoporosis and so on);
— correspondence of the food chemical composition to the age-related changes in metabolism and functions;
— balance of diets by all essential factors of nutrition;
— alkaline orientation of nutrition to correct acidotic characteristics of homeostasis (acidification of the body internal environment) that develop in the old age;
— enrichment of diets with products and dishes that normalize intestinal microflora of the ageing body;

— enrichment of food with substances having geroprotective properties;
— the use of foods and dishes that are quite easily subjected to the action of digestive enzymes and assimilation processes;
— the amino acid composition of foods should correspond to the ideal protein FAO/WHO;
— the mass faction of amino acid tryptophan should be not less than 1 g in 100 g of protein;
— the mass fraction of lysine relative to the mass fraction of methionine + cystine should tend to one;
— the ratio of mass fractions of saturated, monounsaturated and polyunsaturated fatty acids should correspond to the ratio of 3:6:1; with that, the set of polyunsaturated fatty acids should include the acids belonging to the ω-3 group (linoleic, linolenic, arachidonic acids), which are crucial components in treatment and prophylaxis of cardiovascular diseases, as they can prevent the formation of cholesterol and triglycerides in blood, increase resistance of the body to infectious diseases in combination with such amino acids as arginine and glutamine, improve renal function, alleviate inflammation processes in the intestine and joints;
— the ratio of the protein mass fraction to the lipid mass fraction should be 1:0.8;
— the energy value of 100 g of a finished product or dish should be in a range of 600–650 kJ;
— products, dishes in a diet should contain vitamins E, C, PP and B group, which presence in a product facilitate retardation of the ageing process; minerals — potassium, calcium, magnesium, phosphorus, iron, selenium, zinc, as well as components that inhibit processes of lipid membrane oxidation, stimulate peristalsis and facilitate regulation of cholesterol metabolism.

A special role in developing a diet for the elderly is assigned to the selection of products and dishes. All of them should be finely dispersed, their consistency should be tender. It is associated with the difficult process of digestion and assimilation in the gastrointestinal tract of the elderly.

In this connection, a choice of a product type from an assortment line of meat products is extremely important when developing meat gerodietetic products. On this basis, paste is most suitable among all types of meat products.

After choosing a product type it is necessary to determine its purpose: in our specific case it is a product intended for dietetic nutrition aimed at reducing risks of development and prophylaxis of hyperlipidemia and atherosclerosis.

When solving the set task, it is necessary to choose correctly raw material sources. Having the above mentioned data and based on the database of raw material composition, it is possible to formalize the requirements to the product composition.

The restricting parameters for *meat raw material* were the protein content not less than 18% and fat content not higher than 15%; the presence of tissue specific peptides with the molecular weights of 809.4 ± 1.0; 776.5 ± 1.0; 765.6 ± 1.0; 739.2 ± 1.0; 710.8 ± 1.0; 229.2 ± 1.0; 162.1 ± 1.0; 156.0 ± 1.0; 148.1 ± 1.0; 140.2 ± 1.0 and 133.1 ± 1.0 Da; the presence of Ano 1 (takes part in formation of high-density lipoproteins), which can be confirmed by the presence on an electrophoregram of the stained spot with characteristics of 25.0/4.95 Mm (kDa)/pl (or calculated value of 30.3/5.38 Mm (kDa)/pl) or the presence of pre- Ano A-1 (takes part in inhibition of oxidative stress) confirmed by the presence on an electrophoregram of the stained spot with characteristics of 25.0/5.0 Mm (kDa)/pl (or calculated value of 30.0/5.47 Mm (kDa)/pl); for *plant raw material* — the protein content not less than 7% and fat content not more than 6%.

As a result of the work with the database for a product under design, the *porcine aorta*, which contains 22 tissue-specific peptides with the molecular weight of up to 2000 Da including biomarkers apolipoprotein A1 and peroxiredoxin, was chosen as a main raw material.

Then, it is necessary to select the mass fractions of chosen recipe components (porcine aorta, porcine heart, potato starch) in such a way that the finished product corresponds to the requirements for gerodietetic products.

In the process of modeling, a range of variation of the mass fraction of each recipe ingredient is determined so that the obtained product levels the pathological processes and reduces a risk of the development of hyperlipidemia and atherosclerosis and also could not negatively affect the organoleptic properties of the finished product (at this stage, the targeted function is also determined).

As paste was chosen from the available range of meat products, the traditional specific functional and organoleptic characteristics (tender, pasty-like consistency), as well as an effect of the technological process stage in its preparation on changes in the composition and properties of raw materials (the structure, structural-mechanical characteristics, ratio of moisture, protein and fat) should be taken into consideration in calculation of the recipe composition.

The task of structural-parametric optimization of a multicomponent product in different settings and combinations of linear and non-linear criteria and constraints is solved by simulation modeling using all possible combinations of the initial recipe components with the following verification of constraints and calculation of criteria by the algorithm presented in Figure 4.

A search for an optimal composition of an individual functional or specialized food product from *m*-components begins from selecting the mass fraction of the first component X1 and specifying the initial values nj=n0 of the Kj ratio factors (j=2, m-1), that determine the proportion of the jth component in the total mass fraction of the components j, j+1, …, m. The zero value Kj=0 means an absence of the jth component in the remaining mixture, and Kj=1, respectively, means an absence of all other components except the jth.

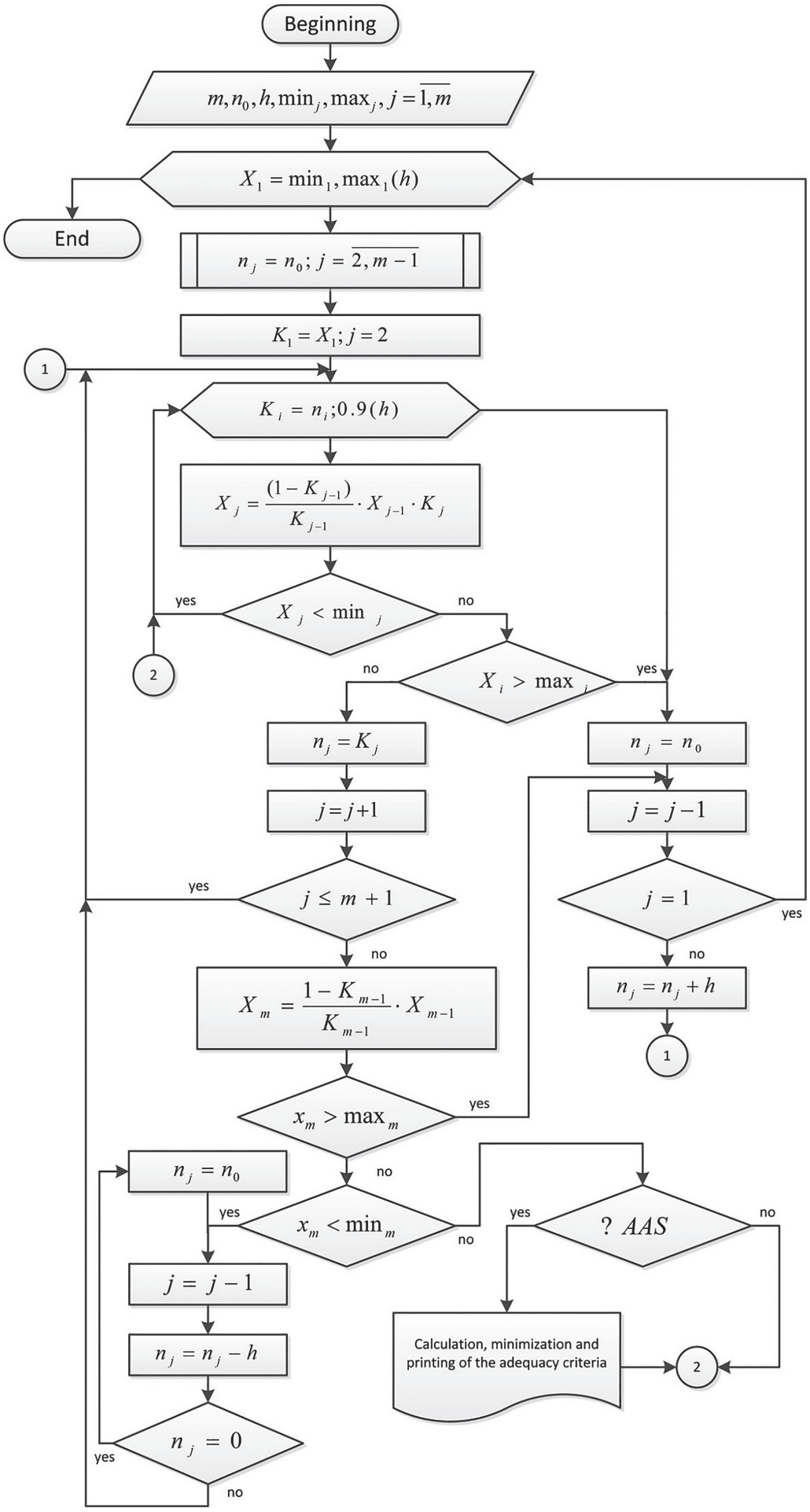

Figure 4. Algorithm of structural-parametric optimization of m-component foods by the method of simulation modeling

Therefore, when presetting a cycle of the trial of the Kj ratio factors (j=2, m-1) from some initial value nj=n0 to the final 0.9 in increments of h, all combination variants of a recipe of the mth component product are simulated with determination of the mass fraction of the jth component Xj by the recursive equation

$$x_j = \frac{(1 - K_{j-1})}{K_{j-1}} \cdot X_{j-1} \cdot K_j, \quad j = \overline{2, m-1} \qquad 1$$

Then, if Xj satisfies boundary conditions, the memorization of the next ratio nj=Kj and transfer to the cycle of the next Kj ratio for the j=j+1th component is followed. When Xj<minj (does not enter the established range of variations), the coefficient of its share participation with the following components is increased by a value of the increment h, and in the case of Xj>maxj (exits beyond the established range), there is a return to the initial jth ratio nj=n0 and continuation of the previous cycle by Kj for the j=j-1th component with the start value nj=nj+h. Similarly, the constraints for the last component at j=m and Km=1 are checked.

After ratios for all recipe components are identified and the mass fractions in total are 1 (one), the parametric and balance constraints with detection of one of the allowable variants of the area of allowable solutions are verified.

As a result of calculations by the method of simulation modeling with consideration for the above mentioned, the distribution of the mass fractions of recipe ingredients with corresponding assessment of the nutritional and biological value of product forcemeat as well as assessment of geroprotective properties are obtained.

Therefore, computer modeling of a diet and food product allows:

1 Assessing actual nutrition (revealing risks of diabetes, excessive weight, cardiovascular diseases and so on);
2) Detecting controlled indicators, which is necessary to carefully monitor (diabetes — the level of glucose, excessive weight — body mass, body mass index, cardiovascular diseases — atherogenicity index, blood pressure and so on);
3) Establishing an individual norm of nutrient consumption based on recording information about physiological parameters, physical and psychological burden, risk or presence of chronic diseases, ecological conditions, habits and lifestyle (parametric description of an individual);
4) Modeling (designing) optimal curative, heath improving, prophylactic and geroprotective diets and food products, corresponding to an individual norm;
5) fulfilling prescriptions: monitoring of controlled parameters, as well as orders regarding a diet, physical activity, physiological status and so on;
6) giving recommendations on a weight change, using BAAs and other means for correction of diet deficiencies [10].

Conclusion

The studies show that spread of diseases, which risks are directly linked with malnutrition (excessive body weight and obesity, disorders of reproductive health as well as cardiovascular diseases, diabetes mellitus, osteoporosis, several malignant neoplasms and others) require a lot of attention to the problem of adequate nutrition. Cardiovascular diseases occupy a special place among these diseases. Prophylaxis is associated with the development of the individual functional foods. There is no question that modern technologies allow accounting for a large number of input parameters with consideration for metabolism of nutrients when modeling products.

This work presents the methodology of structural-parametric modeling, which allows designing personified optimal nutrition for individuals based on their medical indicators in terms of minimization of the risk function.

The choice and substantiation of the recipe composition is shown by the example of a meat gerodietetic product and is based on the principles of structural-parametric modeling.

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Original scientific paper

THE STUDY OF THERMAL DENATURATION OF BEEF, PORK, CHICKEN AND TURKEY MUSCLE PROTEINS USING DIFFERENTIAL SCANNING CALORIMETRY

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Key words: *DSC, protein denaturation, effective specific heat, pork, beef, chicken, turkey*

Abstract

In the temperature range from 45 °C to 90 °C the process of thermal denaturation of a whole complex of muscle proteins in meat takes place. An effective mode to register the thermal denaturation process is the method of differential scanning calorimetry (DSC). As a result of studies the differences during the process of thermal denaturation of muscle proteins of pork, beef, chicken and turkey were defined by the appearance of endothermic peaks in DSC thermograms. The main variances are associated with the process of denaturation of myosin and sacroplasmic proteins and indicate indirectly their quantitative ratio in meat. The values of effective specific heat capacity in the temperature range from 20 °C to 90 °C are obtained as well as those of heat spent on the denaturation process.

*At reheating, the values of specific heat capacity increased by 0.1 J/(g*K) on the average, and peaks of thermal denaturation were not detected, that certifies the irreversibility of the denaturation process and the decrease in the bound moisture proportion in meat after thermal processing. Knowledge of the nature of protein thermal denaturation of each kind of meat product is one of the necessary tools for developing the technology of meat product thermal processing.*

Introduction

During thermal processing reversible and irreversible physical and chemical processes occur in meat products, due to that they acquire necessary taste and food digestibility increases [1]. But under the impact of high temperatures the negative changes take place too, such as contraction and compaction of muscle fibers, juice release that lead to loss of vitamins and precious microelements [2].

A lot of scientific works of domestic and foreign scientists are devoted to the study of thermal protein denaturation [2,3,4,5,6,7,8]. The major methods applied are differential scanning calorimetry and electrophoresis. The differential scanning calorimetry (DSC) is the most straightforward procedure and effective method to register the process of protein denaturation [8]. This method fits well for analyzing food systems often subjected to heating and cooling during technological processing [6].

But the initial DSC studies of denaturation of different types of muscle protein gave discrepant results [9,10,11]. It was due to one or combination of next variables: pH, muscle type, connective tissue, thermostability of muscle proteins. Post hoc, the attempts were made to normalize the variations by detailed study of the impact of each factor. Stabursvik and Martens [12] have developed a method to obtain reproducible curves by pH correction and removing sarcoplasmic and connecting proteins without the need to separate individual proteins for analysis.

From literature data it is known [5] that the thermal denaturation of animal muscular tissue protein begins at temperature above 40 °C and lasts step-by-step up to 125 °C. Herewith, three major transitions are defined that are reflected in the form of peaks in the DSC thermograms: in the temperature range from 50 °C to 65 °C the transition is associated with myosin denaturation; from 60 °C to 75 °C with denaturation of sarcoplasmic proteins and connected tissue; with denaturation of different forms of actin in the temperature range from 71 °C to 83 °C) [5,7,9,12].

In the work [13] the studies of the influence of the thermal processing temperature on the degree of denaturation of myofibrillar and sarcoplasmic protein fractions of pork of NOR and PSE qualitative groups were carried out using electrophoresis. A marked dependence of the intensity of denaturation of sarcoplasmic proteins on the pork meat belonging to one or another qualitative group was revealed. At the same time the nature of denaturation of myofibrillar proteins of NOR and PSE pork was identical.

The addition of various recipe ingredients in meat raw materials also affects the process of thermal denaturation of proteins. DSC studies of the effect of mono- and divalent salts on the protein thermal stability are given in works [14,15,16,17]. The studies of each work were carried out using meat produce of certain species of animals, mainly pork and chicken.

This paper reports the results of studies of muscular tissue of pork and beef meat, chicken and turkey meat performed using DSC method in order to compare the behavior of thermal denaturation process in the connection with belonging of meat to one or another animal species. The thermograms of muscular tissue of different animal meat and poultry were compared; the changes of specific heat capacity before and after thermal processing were analyzed as well.

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Establishing the relations between biochemical factors and the character of thermograms of raw materials at different protein content is promising. Today, there is still a lack of researches in this field. To provide a high quality meat products it is needed to define the individual thermal processing conditions on the basis of knowledge of thermal denaturation of each kind of meat raw material. That will provide quality increasing of an output product.

Materials and methods

The samples of muscular tissue of the longissimus dorsi muscle of pork and beef, sternum muscle of chicken and turkey were selected for studying. Three samples of each meat were selected; they were muscular tissues without skin, streaks of fat and bones. Sample mass was from 200 to 300 grams. The muscular tissue samples were purchased in big department stores of known trade networks.

During experiments three measurements of each meat were carried out.

The NETZSCH DSC204 F1 device (NETZSCH-Geratebau GmbH, Germany, 54912–13 number in the State register of approved types of measuring apparatuses) was used for measurements. According to instrument rating a relative error was no more than ±2.5% and ±3.0% respectively at definition of specific isobar heat capacity and phase transition enthalpy.

The calorimeter was calibrated for temperature and sensitivity on the basis of the set of standard substances "from minus 64.5 to +476 °C" (according to producer's instructions). The set includes C10H16 (minus 64.5 °C), In (156.6 °C), Sn (231.9 °C), Bi (271.4 °C), Zn (419.5 °C), CsCl (476.0 °C). Also the additional distilled water temperature point (0 °C) was used. Calibration points were reproduced at accuracy of ±0.1 °C.

During studies the discs at mass of 20–30 mg, corresponding to the inner diameter of a standard aluminum crucible (V = 25 μl, d = 6 mm), were cut from the muscular tissue samples.

Then, the sample was placed in the pre-weighted crucible, covered with a lid, pressed in and reweighted. The weighting was carried out using a laboratory balance MB 210 — A. According to instrument rating the root-mean-square deviation of balance reading was 0.03 mg.

The DSC temperature research program, developed by the authors, includes the following stages: cooling to minus 5 °C followed by maintaining during 4 minutes to stabilize the temperature of the measuring cell and the test sample; dynamic stage of heating from minus 5 °C to 90 °C at 10 K/min speed and the final stage — cooling to a room temperature.

The study of one sample was carried out twice consecutively in the same crucible. Thus, we obtained the DSC thermograms and the values of the effective heat capacity of muscular tissue of animals and poultry (pork, beef, chicken, turkey) in raw form and after heating, calculated by the method of relations [19,20].

Results and discussion

When heating the samples of raw muscular tissue of pork, beef, chicken and turkey the strongly pronounced endothermic peaks are observed that reflect the heat absorption during protein thermal denaturation (Figure 1a). In previously published works [5] the generalized data are usually given without specifying the species of animals and muscle groups. Figure 1 clearly shows that the thermograms of each animal species have their own unique character due to meat composition and structure. At reheating the DSC curves are of increasing character without strongly pronounced jumps that is due to irreversibility of the denaturation process (Figure 1b).

Figure 2 presents the diagram with generalized data on the temperature range of denaturation of main muscle protein types obtained from published works [1,5,7,14,16,17,20]. The diagram shows that in the temperature range from 50 °C to 70 °C the denaturation of six protein types takes place simultaneously that is demonstrated in the DSC thermogram as a sum of peaks. For all meat samples the most pronounced peak is the last one, reaching a maximal value at 80 ± 2 °C temperature and showing the process of actin and globulin–X denaturation.

Calculated by the method of relations the values of effective specific capacity of raw muscular tissue of test samples and their values at reheating are shown in Figures 3, 4, 5, 6.

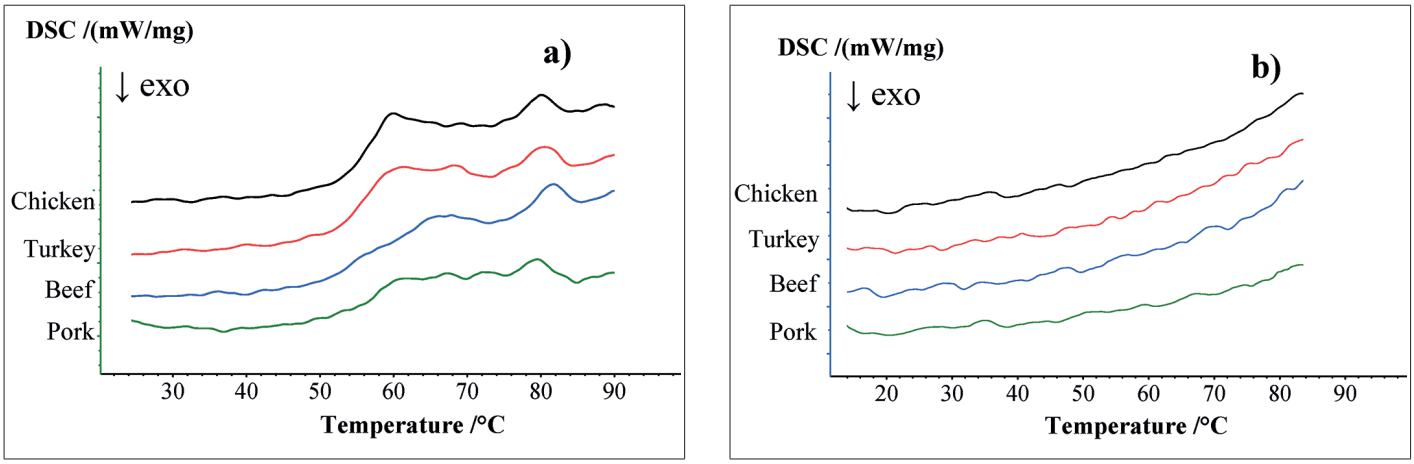

Figure 1. DSC thermograms at heating: a) raw meat samples; b) sample reheating

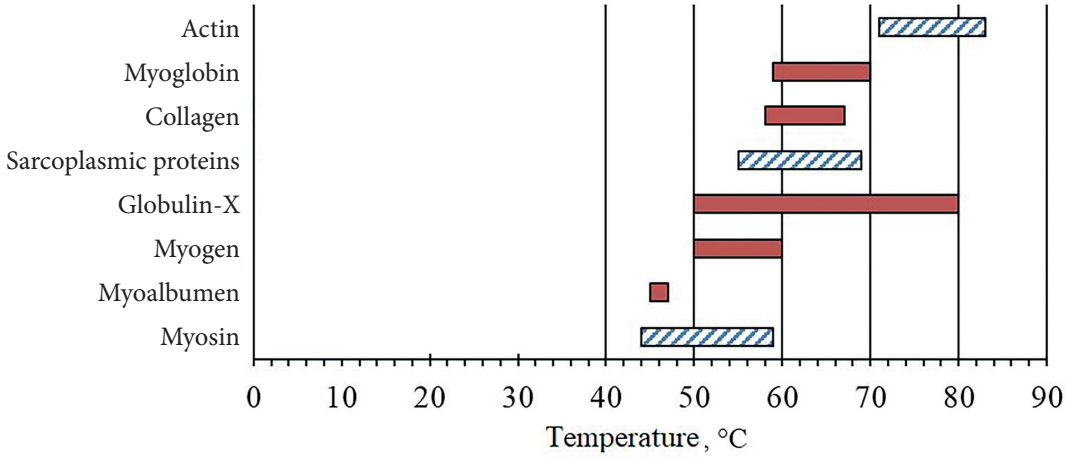

Figure 2. Denaturation temperature range of main proteins according to data [1,5,7,14,16,17,20]

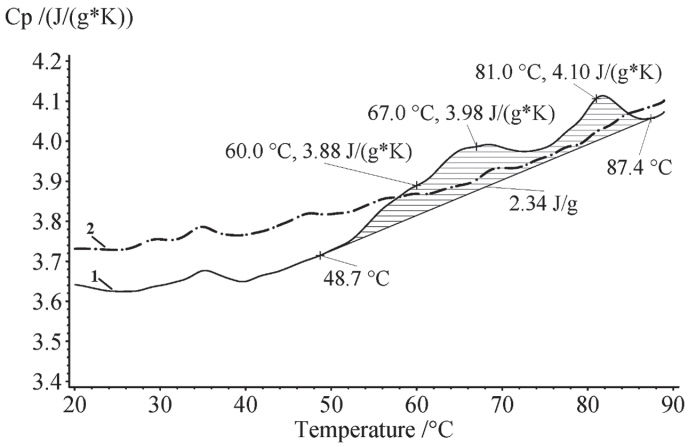

Figure 3. Beef specific heat capacity in native (1) and denatured form (2)

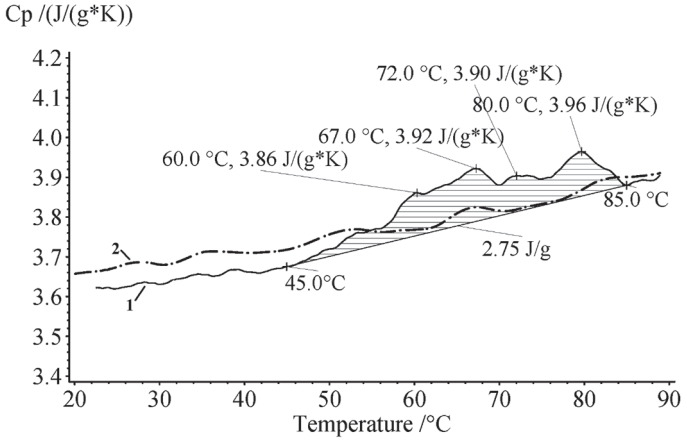

Figure 4. Pork specific heat capacity in native (1) and denatured form (2)

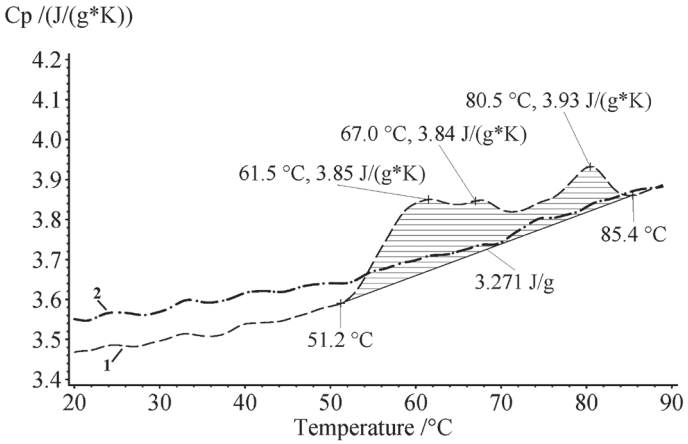

Figure 5. Turkey specific heat capacity in native (1) and denatured form (2)

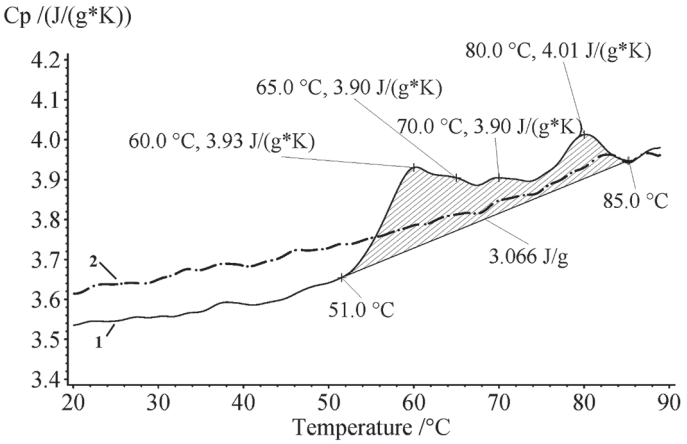

Figure 6. Chicken specific heat capacity in native (1) and denatured form (2)

According to the data obtained previously [14] three peaks of denaturation are distinguished (myosin, sarcoplasmic protein, actin); however, the first beef muscular tissue peak at 60 °C is latent and joins the next, that is maximal at 67 °C. For pork four equivalently pronounced peaks are typical at 60 °C, 67 °C, 72 °C and 80 °C temperatures respectively. For chicken and turkey samples the first and the last peaks are more pronounced with maximum values at 60 ± 2 °C and 80 ± 1 °C.

The data of chicken meat thermograms are well correlated with the results of work [17], in which a highest evidence of the first and the last peaks of denaturation as well as less pronounced intermediate ones are shown.

Also it was defined that the denaturation process of pork and beef samples began at lower temperatures (45 ± 1 °C and 48 ± 1 °C respectively) in comparison with chicken and turkey meat (51 ± 1 °C). The process ended at 85 ± 1 °C temperature in all kind of meat, except beef meat, the denaturation of which occurred at 87 ± 1 °C temperature.

The data obtained certify the denaturation process uniqueness for each kind of meat raw material. As shown in works [5,14], the denaturation peaks shift in the presence of salts in

meat. There are sufficient reasons for extending researches as regards the influence of different additives on various animal meats. The accumulation of data on denaturation process behavior is needed for technology development as well as for improving the control of thermal treatment processes.

At samples reheating the denaturation peaks are not detected as it is seen in diagrams 3, 4, 5, 6, and the values of the sample specific heat capacity obtained in denatured form are higher by 0.1 J/(g*K) as compared with those in native form. It is due to:

— irreversible changes of muscle fiber structure provoking fiber contraction and meat juice release that result in decrease of the proportion of bound moisture;
— in accordance with the experimental research program, the juice released remained with the meat sample in a sealed crucible at reheating. It allowed comparing the values of the sample specific heat capacity in native form with those of the denatured one at identical values of moisture and mass content.

The thermal properties of meat raw materials are needed as the initial data for calculation and modeling the processes of thermal treatment. In reference sources [18,19] the values of specific heat capacity of meat raw materials are presented fragmentarily, and as a rule, at temperatures above freezing they are accepted as constants and averaged ones. It advisable to take into account an increasing character of changes of specific heat capacity values as well as the need to spend additional energy on the process of muscle protein denaturation at temperatures above 45 °C.

The maximal values of effective specific heat capacity corresponding to the peaks of protein denaturation in the temperature range from 45 °C to 88 °C for all samples were from 3.85 J/(g*K) to 4.1 J/(g *K).

The heat spent on the denaturation process ranged from 2.3 J/g to 3.3 J/g. Herewith, the higher values were obtained for chicken and turkey samples, but the lowest for beef ones.

Conclusion

At meat thermal processing at temperature above 45 °C an irreversible protein denaturation process takes place. Herewith, simultaneously a complex of different proteins is denatured.

Using the DSC method we showed obviously an individual character of the denaturation process behavior in muscular tissue of various animals and poultry.

The accumulation of knowledge on the nature of thermal protein denaturation of various meat raw materials will make it possible to develop thermal processing conditions providing minimization of the quality loss of a ready meat product.

To decode the peaks of protein denaturation it is necessary to conduct complex researches combining the analysis of DSC thermograms with identification of meat protein composition.

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Review paper

MYOPATHY AS A DESTABILIZING FACTOR OF MEAT QUALITY FORMATION

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Key words: *meat quality, pork, poultry meat, muscle tissue, myopathy, muscle fiber lesions, stress, nutritional myopathy, selenium, vitamin E*

Abstract

This review paper is devoted to myopathy of slaughter animals and poultry, and examines a relationship between fast growth of muscle tissue in hybrid pigs, broiler chickens and turkey, and high frequency of detection of spontaneous or idiopathic myopathies. The development of myopathy reduces consumer and technological properties of meat, and leads to emergence of different pathological conditions (PSE or RSE meat, «destructured meat», «white» or «green» meat, punctate hemorrhage, «wooden breast» and others). Two types of myopathic conditions are examined: myopathies caused by stress in animals and nutritional myopathies, which contribution to meat quality deterioration seems to be determinative. It is shown that the basis of the mechanism of the myopathy development is the mechanism of the successive changes in muscle tissue: damage of cell membranes and release of mitochondrial calcium, which causes hypercontraction, dystrophic changes, atrophy and necrosis of muscle fibers. To alleviate the damaging effect of two types of myopathies, different substances-adaptogens (selenium, vitamin E, flavonoids and others) can be used. It is stated that the requirements of animals in adaptogens change with an increase in the indicators of their productivity.

Introduction

Over the last decades, the agriculture has achieved an exceptional success with regard to the growth of the efficiency indicators in beef husbandry and poultry production, including an increase in the rates of muscle tissue gain in feeding as well as the rise in the mass fraction of muscle tissue in the body of slaughter animals and poultry. For example, over the last 50 years, the size and live weight of chickens and turkey changed by two times mainly due to the content of pectoral muscles; while in pork carcasses, the content of muscle tissue increased from 44–49% to 58–62% during the same period [1,2].

However, this success has some negative consequences. The consumer and technological properties of meat quality have changed. The problem of a decrease in meat quality and prevalence of specific defects in meat and meat products associated with myopathy has been discussed more and more often in foreign and domestic practice. After multiple studies, the scientists formed an opinion that the genetic progress enhanced a burden on fast-growing slaughter animals and poultry, and led to the morphological and biochemical modifications of muscle tissue that deteriorated meat consumer properties. The performed studies show that fast-growing hybrids demonstrate high frequency of detection of spontaneous or idiopathic myopathies, including those that are caused by stress, which are of great importance for meat quality and result in emergence of different pathological conditions (for example, PSE meat). Simultaneously, economic importance of meat quality questions both for consumers and meat processors is more and more actively recognized worldwide [1].

Muscle tissue, which is the major part of the animal body, is considered the main component taking part in meat quality formation [2]. In this connection, the study of myopathic conditions caused by feeding and stress in animals is of special interest in the context of lifetime formation of meat quality. This paper presents the review information on the results of the foreign research in the field of myopathy investigation and their effect on meat quality with the aim of generalization and analysis of modern scientific knowledge on this question, and determination of the main myopathy types as the most destabilizing factors of lifetime meat quality formation in fast-growing animals and poultry.

Main part

Muscle tissue damage caused by myopathy

Myopathy (Greek: mys, my[os] muscle + pathos: suffering, disease) is a progressive degenerating neuromuscular disease, in which the metabolism disorder occurs leading to a reduction of the muscle tonus, damage of muscle fibers with their following atrophy. Forms of myopathy and its types can be different. Among them are genetically conditioned (inherited and innate) myopathies, in which a gene defect that caused a disease is known; myopathies of the metabolic character (as a rule, they are also inherited), toxic myopathies; infectious myopathies; nutritional myopathies and other myopathies, including myopathies of unknown etiology [3,4].

All species of mammals including humans are subjected to myopathies [5]. Due to similarity of etiology, myopathies in pigs and birds are called an animal model of the human disease [4,6]. Study of the myopathy development

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in animals can be a key to understanding this disease in humans [6,7,8].

Pig husbandry and poultry production have the highest economic losses associated with myopathy. For example, it was estimated that losses due to myopathy in broiler chickens in Poland were 1.2 euro per affected carcass (in prices of 2009). In poultry production enterprises, up to 70% of poultry stock can be affected by myopathy manifested in pectoral muscles [9].

Lifetime characteristics of animal skeletal muscles are of fundamental importance for meat quality formation. Their diversity is enormous taking into consideration the fact that skeletal musculature of an animal includes about 570 muscles with different forms, sizes, location and functions. With that, in general, muscle tissue directly participates in formation of meat tenderness, juiciness and color, and also determines its moisture holding capacity in the raw and finished (processed) form. Its characteristics contribute to a significant variability of meat quality parameters [2,10].

It is known that muscles consist of muscle fibers with different sizes (length and diameter). A fiber diameter varies within a muscle and between muscles, depends on age, physical load, nutrition state and animal species. Muscle fibers in external muscles always have a low diameter (10–30 μm); on the contrary, a diameter of the main muscles of limbs can achieve significant sizes (40–65 μm). A fiber size increases with age until sexual maturation. A fiber diameter is slightly larger in males than in females. It can depend on feeding: according to data [2], the positive genetic and phenotypic correlation between the intramuscular fat content and a muscle fiber cross-sectional area is observed in pigs. In the old age, a fiber diameter slowly decreases. However, as a result of diseases, pregnancy and/or nutrition state, a fiber diameter can change [3]. Along with average muscle fiber sizes, a ratio of muscle fiber types plays an important role in meat quality formation.

Type II fibers (white, fast, glycolytic) are more sensitive to the development of pathological conditions compared to type I fibers (red, slow, oxidative). Therefore, the more such fibers in a muscle, the higher the probability of the development of myopathy. Selection studies aimed at increasing the content of type I muscle fibers show the possibility of meat quality improvement [10,11]. The highest content of type II fibers is typical for muscles *m. Longissimus, m. Psoas and m. Semitendinosus.* In this connection, it is recommended to send them to histological investigations to diagnose myopathic conditions [3,12,13].

Muscle fibers are dynamic structures, which upon certain conditions can turn from one type to another according to the transition scheme: type I ↔ type IIA ↔ type IIX ↔ type IIB* [2]. The predominant type of muscle fibers depends on many factors — muscle function, animal species, breed, age, physical activity, ambient temperature and nutritional behavior. For example, pork skeletal muscles show an inclination to an increased content of type IIB muscle fibers [14]. Pork *m. Longissimus dorsi* contains about 10% of type I fibers, 10% of type IIA, 25% of type IIX and 55% of type IIB [2]. With that, the content of type 2B muscle fibers in *m. Longissimus dorsi* can reach more than 90%, which is a result of selection [15].

The total fat content in muscles positively correlates with the content of oxidative fibers and negatively correlates with the content of glycolytic fibers. For example, in pigs, the mass fraction of fat can be up to three times higher in the white part of *m. Semitendinosus* than in the red part of this muscle [16]. At the same time, other studies suggest an absence of strict relationship between the total content of intramuscular fat and a ratio of fiber types in muscles [17].

Fibers of the glycolytic type are characterized by larger diameter compared to oxidative and intermediate fibers irrespective of animal age.

A local increase in a muscle fiber diameter characteristic of glycolytic fibers is revealed in muscle tissue after slaughter as hypertonic giant fibers. This defect is typical for PSE meat and especially often is seen in poultry and pork. It is directly linked with poor quality meat. Muscle tissue predisposition for the post mortem development of giant fibers («nodes of hypercontraction») is formed lifetime and can also be diagnosed lifetime by skeletal muscle biopsy with the following sample incubation at 37 °C for 60 min [11,18].

Skeletal muscles are prone to atrophy and many other degenerative lesions beginning from focal ruptures of sarcolemma and ending with necrosis. Necrosis can vary by the severity degree from several necrotic fibers to gross lesions of muscles. The unique peculiarity of necrosis in fibers of skeletal muscles is the fact that it can be segmental affecting only part of a fiber, which is a consequence of multinuclear nature of muscle cells. In the necrotic segments of muscle fibers, myofibrils and other cytoplasmic structures and sarcolemma are subjected to lysis; however, the basal plate and endomysium are often undamaged [3].

The main cause of atrophy is undernutrition and/or insufficient intake of certain nutrients with feed. When nutrients are deficient, muscle proteins can be mobilized as their source for the whole body. Muscle tissue atrophy is the most prevalent damage of skeletal muscles and can be observed in different types of myopathy. With that, an outcome of atrophy can be degenerative changes in muscle tissue up to coagulative necrosis [3].

Pathogenesis of different myopathies, including nutritional, is characterized by a specific sequence. As muscle necrosis leads to damage and finally destruction of sarcolemma of fibers, normal ionic gradients cannot be maintained. Calcium ions, which are usually in lower concentration in cytoplasm than in the extracellular fluid, diffuse into a cell. Entry of calcium ions leads to activation of calcium-dependent proteases, such as calpains, and the following destruction of myofibrils and other structural elements of a muscle fiber. Hence, in muscle tissue, like in other tissues, there is a successive common destructive pathway of overloading of mitochondrial

* In addition to two main types of muscle fibers, there are five intermediate types.

calcium, which begins as a result of membrane or energy deficiency and ends with coagulation and dissolution of contractile proteins. Histologically, an increase in the content of calcium ions taken from blood is observed in muscle fiber sarcoplasm; with that, blood supply of a damaged muscle is not disturbed. An increase in the concentration of calcium ions in sarcoplasm determines formation of areas of myofibril supercontraction in the structure of muscle fibers. A change in homeostasis results in destruction of cytoskeletal proteins, damage of the structure of cell organelles and their necrosis in addition to the development of contractures of myofibrillar structures. Due to color changes in damaged areas of a muscle, myopathies with the severe form of necrosis are often called «white muscle disease»**. It is also worth noting, that less severe forms of muscle necrosis, in which smaller part of fibers is affected, is often difficult to detect upon general visual examination. However, they undoubtedly play a role in meat quality formation [3].

Myopathy is diagnosed most easily by the method of macroscopic examination in poultry after slaughter. The disease is manifested above all by changes in color (from pink to red or green depending on the degree of the development) and texture (increased hardness and fibrousness) of affected muscles [9].

Microscopic investigation of animal muscle tissue can detect damaged and even necrotic fibers. Changes in muscle fibers can be widely distributed and characterized as multifocal monophasic or even polyphasic lesions. This can point to recent nonfatal episodes of myopathy or to chronic pathology [3].

At the end of the 1990s, observations and descriptions of the defect of pork muscle tissue, which was caused by myopathy and called «destructured meat», appeared. The defect was manifested only in pork hams. The color of affected muscles was strongly altered: meat looked very pale and greyish. Colormetric investigations showed that the values L (lightness), a (redness) and b (yellowness) increased in muscles depending on how much they were affected by this defect. Highly destructured muscles lost their organized structure. They had higher glycolytic potential compared to unaffected muscles. Histological analysis revealed similarity of muscle destruction with the PSE defect, which was manifested as an increase in the interfibrillar space and supercontraction of muscle fibers [19].

Emergence of «destructured meat» led to significant losses in production of cooked hams (grey color untypical for meat products produced with sodium nitrite, the unbound and fragmented structure of the finished product, the presence of holes, bad consistency and slicing) [19].

To classify a level of ham muscle damage by this defect, the following gradation of meat after boning and trimming was proposed: 1 — ham muscles do not have the «destructured muscles» defect; 2 — the defect is seen, superficial, located only in the semimembranosus muscle; 3 — the semimembranosus muscle is strongly altered and the defect partly affects adjacent muscles; 4 — ham muscles are destructured (destroyed) [19].

The large-scale investigations carried out in five commercial slaughterhouses in France (more than six thousand pork carcasses), allowed assessing the prevalence of «destructured meat» — the defect seriously or fully affected ham muscles in up to 17% of pork carcasses. With that, more than 80% of hams with this defect had the pH value of aged meat lower than 5.60. Later on, the low pH value was recognized as the main risk factor for emergence of this defect. Also, the backfat depth, leanness and carcass weight of an animal were assigned to the risk factors [19,20]. At the same time, «destructured meat» cannot be regarded as the PSE defect. Maximum quantity of carcasses with destructured ham muscles was revealed at pH 5.5–5.6 (44.9%). At pH lower than 5.5, the proportion of carcasses with the defect reduced (up to 32.5%). In addition, an effect of gender, stress, genetics, maturity of collagen in muscle tissue was evaluated; however, causes leading to this defect still are not fully revealed. Finally, the defect was called a «disease of thin piglets» as this defect appeared in large quantities in enterprises that turned to raising pigs with backfat depth less than 14 mm [19,20, 21]. In more recent studies of pork muscles, an association was found between emergence of defects of the «destructured meat» type and the prevalence of type 2B fibers [12,13].

Myopathy in chickens is known under the names: deep pectoral myopathy, green muscle disease, Oregon disease or degenerative myopathy [4,6]. It is ischemic, spontaneous necrosis, which in chickens affects mainly the pectoralis minor muscle (m. Pectoralis minor) and leads to changes in color and texture of muscle tissue. Two stages are recognized in poultry myopathy progression — early and late. At the early stage, a muscle has characteristic reddish or pink hemorrhages; later on, muscle tissue becomes green or pale grey and contracts; at the micro level, multiple nodes of contraction are formed [6,9]. By symptom classification, three phases are recognized in poultry myopathy: the first phase — an acute inflammatory lesion with multiple hemorrhages, the second phase — a muscle becomes pale and resembles «fish flesh»; the third phase — the progressive degeneration with green necrotic areas [23].

The etiology of the disease in poultry (chickens and turkey) as well as in pigs is not fully revealed. It is believed that the main reason is the fact that selection in meat breeds of poultry was focused on growth rate, musculature and feed conversion (pectoral muscle degeneration is not observed in wild birds). An increase in the muscle fiber diameter leads to a decrease in the free space for connective tissue, reduction of blood supply and disorder of metabolism in pectoral muscles. These changes constrain the mechanisms of muscle recovery, which, apparently, provoke appearance of myopathy. Moreover, the development of myopathy in the pectoralis minor muscle is influenced by its characteristic anatomical location, which prevents hypertrophy of muscle mass upon

** Nutritional myopathy is also described in scientific literature under the names «nutritional myodegeneration», «nutritional muscle dystrophy», «lamb disease» and others.

physical load, causing occlusion of blood vessels and inducing tissue necrosis. Along with the pectoralis minor muscle, the pectoralis major muscle can also be affected [9, 23, 24].

In fast-growing broiler chickens, myopathy was described as a defect, which was given a name «wooden breast» [25]. Macroscopically, the affected pectoralis major muscle is hard, pale, with the

presence of bulges, sometimes is superficially covered with small hemorrhages, exudate and occasionally has characteristic white striping; affected parts also show extended areas with poor binding of muscle bundles [26]. Histologically, the condition was determined as moderate or severe polyphasic myodegeneration with regeneration of muscle tissue. With this defect, inflammation and necrosis are accompanied by accumulation of interstitial connective tissue (fibrosis), which explains an increase in hardness of the affected tissue as a result of an increase in the content of intramuscular collagen [27]. Moreover, upon muscle damage, there is an increase in an amount of extramyofibrillar water, which is significantly lost over time leading to an increase in meat hardness. With that, affected muscles can show heterogeneity of structural-mechanical properties in different layers [28].

Therefore, the study of myopathic conditions demonstrates that primarily animals that can gain muscle mass quickly are prone to the emergence and development of the disease. With that, microstructural changes in muscle tissue inevitably lead to meat quality deterioration and loss of meat functional-technological and consumer properties.

Myopathies caused by stress in animals

Formation of meat quality takes place as a result of post mortem evolution of animal muscles into meat and depends not only on biological characteristics of muscles, but also on the stress level in animals before slaughter [2].

Stress can cause muscle dysfunctions and dystrophic changes not only in humans and animals, but even in insects. The studies carried out on Drosophila showed that due to stress damaged muscles were less adaptive, more sensitive to energetic stress and to changes in the ambient temperature [29,30].

Stress in farm animals is the main cause of myopathies and meat quality deterioration. With that, the problem still has such a scope that there is a real danger that consumers can begin associate low meat quality with the problems of meat product safety in general [31]. It is known that stress leads to emergence of meat with PSE (pale, soft, exudative) and DFD (dark, firm and dry) defects [32]. Over the last decades, other degrees of meat quality deterioration have been distinguished such as RSE (red, soft and exudative) and RFN (red, firm and non-exudative). For example, RSE pork is characterized by red color as in «normal» pork (RNF), but shows properties of exudative PSE pork [33].

In pork production, the proportion of meat with reduced quality resulting from stress before slaughter ranges from 10% to 30%, and in several countries up to 60%. As extreme clinical signs, porcine stress syndrome (PSS) is manifested as paralysis of the heart and necrosis of *m. longissimus dorsi*. As a result of movements, overheating, overstocking and other factors, the content of myoplasmic calcium increases in porcine blood and tissues. In the USA, up to 8% of pork carcasses are rejected due to myopathy caused by PSS [31,34].

It is believed that a cause of wide distribution of PSS was parents heterozygous by mutation in ryr 1 gene. This gene is responsible for synthesis of ryanodine receptor protein, which is found in the sarcoplasmic reticulum of a muscle fiber. The main function of this protein is regulation of the calcium ion concentration in cytoplasm. Even insignificant stress impact on an animal — carrier of this mutation leads to a sharp increase in the content of calcium ions in sarcoplasm, as a result of which muscle fibers begin to work in the regime of the extremely enhanced muscular load. A rise in myoplasmic calcium (Ca2+) is accompanied by an increase in heat production due to activation of phosphorylase and breakdown of ATP, glycogenolysis is enhanced with production of lactic acid, CO2 and excess of heat. An ATP deficiency is created, which leads to a damage of the actin-myosin complex, emergence of supercontractions of myofibrillar structures of muscle fibers and fast muscle rigidity. Blood pH drops and metabolic acidosis develops. Upon the severe form of PSS, an animal, as a rule, dies. Mild forms of the disease are latent and are detected only during carcass cutting, when the so-called «white muscles» (visually revealed areas or whole muscles that are distinguished by paleness and wateriness) are observed. In these muscles, macroscopically visible areas with bloody color («punctate hemorrhages») are often found. Histologically, areas with hyaline degeneration and necrosis of muscle fibers are revealed [34].

The first studies on an effect of the halothane sensitivity gene — HAL locus with two alleles N (normal) or n (sensitive) — on zootechnical indicators of pigs go back to the beginning of the 1970s. An interest to n allele was quickly recognized in terms of its influence on meat quality deterioration (a risk of the PSE development). Since 1993, the use of the molecular test has allowed recognizing animals with Nn and NN genotypes as halothane-insensitive pigs and assessing an impact of n allele on productivity of animals and meat quality [21,35,36,37]. This led to the fact that animals — carriers of ryr1 gene with nn alleles were practically excluded from meat production. However, recent studies showed that the work only on one genetic factor ryr 1 did not enable achieving a significant increase in quality of produced pork and reduction of losses. Despite the genetic selection and use of stress-resistant (halothane-negative) animals, the problem remains to be topical. In the practice of pig slaughter and processing, the result of lifetime stress and the development of myopathy in different forms continues to be found in meat quality assessment [34].

Therefore, the development of pig husbandry shows that genetic knowledge is important but it does not make it possible to fully solve the problem of PSS. It was estimated that only 4% of low quality meat was conditioned by genetics

[31]. Pork quality and yield are still influenced by multiple *ante mortem* and *post mortem* factors including transportation and pre-slaughter holding, methods of slaughter and carcass chilling. All this indicate an increased necessity to pay close attention to stability of pork quality and the ways of its improvement [33].

It should be remembered that the age, at which animals are slaughtered and which has been steadily reduced, also makes a contribution in addition to genetics. For example, it was found that the frequency of myopathy detection was influenced by poultry age and weight (positive linear correlation) [9]. Moreover, at present, there is still no verification of the role of genetic mutations in the development of myopathy in chickens and turkey. There are several explanations of the myopathy development that are not connected with genetics, namely:

— Excessive hypertrophy of muscle fibers of the glycolytic type, immaturity of collagen and/or inadequate development of intramuscular connective tissue, disorders of vessels and blood supply of muscles;
— Heat stress — high muscle temperatures due to flapping, struggle, stress, and high metabolic rate in muscles before slaughter (spontaneous or idiopathic, stress-induced myopathy caused by oxidative damage of proteins [38]);
— Large muscle mass of different carcass parts, which is difficult to rapidly chill after slaughter [1], or incubation of muscles at 35 °C [39].

Nevertheless, to prevent PSS, it is necessary to continue creating favorable conditions for animal holding, adhering to the rules of their transportation and the norms of humane slaughter.

Nutritional myopathies

Recently, the term nutritional myopathy appeared in the literature. The majority of researchers link it with deficiency of selenium and vitamin E, as well as with an increased level of oxidative stress emerging on the background of the high animal growth rate.

Nutritional myopathies are largely known as diseases of calves, lambs, pigs and foals. The first clinico-pathological descriptions of nutritional myopathies (by the example of beef cattle) date back to the 1890s; however, this disease was already well known at that time in Germany, France, Switzerland and Scandinavia. Nutritional myopathies are seldom found in predators (this fact clearly indicates once more the benefit of the meat diet) [3].

The most common deficiency in nutrition that leads to nutritional myopathy in the majority of animals is selenium deficiency. Nutritional myopathy caused by deficiency of vitamin E in the absence of selenium deficiency is seldom observed in mammals; however, it can be common in birds and reptiles. On the contrary, deficiency of vitamin E in combination with lowered selenium status, can lead to nutritional myopathy in different species. In this connection, selenium was recognized as an important nutrient and was involved in explanation of causes of nutritional myopathy as far back as the end of the 1950s [3,40].

Nutritional myopathy, like other types of myopathies, can be accompanied with necrosis of muscle fibers. As a rule, it has selective segmentary multi-focal and polyphasic character, leaving the basal plate and satellite cells unaffected, and consequently, ensuring quick and effective regenerative rehabilitation. In extreme cases, nutritional myopathy can be accompanied with myoglobinuria, myocardial damage and rhabdomyolysis. Massive myoglobinuria damaging renal tubules can lead to acute renal failure [3].

Animals with high feed conversion efficiency and significant muscle mass show the highest sensitivity to myopathy and cardiomyopathy caused by selenium deficiency. The problem of such myopathies emerges in different parts of the world not only in animals but also in humans. Selenium deficiency associated human cardiomyopathy named Keshan disease was described for the first time in China as far back as the 1930s [41].

The development of nutritional myopathy is closely correlated with the regions with low (<0.005% of dry substances) concentrations of selenium in plants. Historically, nutritional myopathy is considered a disease of young animals, especially very young. Fast postnatal growth, apparently, predisposes to the problems of nutrient deficiency, although nutritional muscle degeneration is found also in adult animals. At the same time, spontaneous nutritional myopathy can arise prenatally and muscle damages can be observed already at birth. With that, the symptoms of nutritional myopathy can be unobservable in parents (usually, cows or sheep) before or during parturition [3].

One of the most complicated aspects of nutritional myopathies is irregularity and unpredictability of their emergence, especially, in the grazing system. With the same content of alpha-tocopherol, polyunsaturated fats and selenium in pig diets, accumulation of vitamin E and selenium in their organs and muscle tissue can be different. This, in turn, can be associated with the health status of animals, including heart diseases [42].

Therefore, it is agreed that in general the cause of nutritional myopathy is deficiency of one or two substances — selenium and vitamin E. In case of low selenium content in a diet, an increased intake of vitamin E can retard the development of myopathy associated with selenium deficiency [3].

Metabolism of selenium and vitamin E is not fully studied. However, the understanding of factors influencing the integrity of cell membranes and their changes in myopathies led to the understanding of the role of these substances. Enzymes containing vitamin E and selenium are necessary as physiological antagonists of the group of chemically diverse substances known as free radicals. Some of free radicals are endogenous products of normal cell function, others are external and undesirable factors that take part in cell metabolism. They can be produced both in cells and outside cells as products of tissue irradiation, reactions on

pharmaceutical drugs and in inflammations. One of the main sources of free radicals is a process of cell detoxification, which makes substances that enter a cell less harmful transforming them into epoxides. Free radicals can initiate cell damage causing peroxidation of membrane lipids and physico-chemical damage of protein molecules including mitochondria, endoplasmic reticulum and cytosol. Protection against an impact of free radicals is provided by the constant presence of small molecules-scavengers, such as tocopherols, ascorbate, beta-carotene as well as by the presence of selenium-containing enzymes of the system glutathione peroxidase / glutathione reductase. Thus, modern concepts about the development of nutritional myopathy imply that in case of absence of sufficient protection by selenium and/or vitamin E, cell membranes are modified by free radicals. The ability of such membranes to support differential ionic gradients is reduced or lost. This initiates a sequence of events when calcium entry leads to hypercontraction of myofibrils and their necrosis [3,42,43].

In addition to drawbacks of a diet associated with deficiency of selenium and vitamin E, there are several factors that can also lead to the development of nutritional myopathy. Among them are feeding with rancid or oxidized fats; feeding with recently harvested grain; feeding of piglets with pea; the presence of several metals in mineral mixes (supplementary feed) as contaminants (including silver, copper, cadmium, cobalt, vanadium, tellurium, zinc and possibly other metals); copper deficiency, unusual physical load; pharmaceutical drugs; toxic substances in feed; disorders in the thyroid gland function and others [3].

Nutritional myopathy of pigs was observed as a spontaneous disease in all countries with intensive breeding of these animals. Until recently, it was considered that classical lesions of skeletal muscles in pigs upon deficiency of selenium and/or vitamin E occur more rarely than changes in the heart muscle and hepatosis. However, systemic microscopic investigations carried out abroad revealed significantly higher frequency of muscle lesions in pigs than it was thought earlier [3].

In outbreaks of nutritional myopathy, both growing and adult pigs can be affected; with that, nutritional myopathy in the latter can occur without any clinical signs or cause slowness of movements and apathy. Although nutritional myopathy is not considered an innate disease, nevertheless, piglets at the age of 1 day can have gross lesions causing muscle weakness or paresis [3].

In nutritional myopathy in pigs, mineralization of damaged muscle fibers is often not abundant and, even when it occurs and visible, it is difficult to find it in pale porcine muscles. This explains the fact that in nutritional myopathy of pigs, lesions in the heart and liver are found much more frequently; with that, there is relatively a low number of reports about lesions of skeletal muscles. Nevertheless, lesions ranged from microscopic to pronounced macroscopic can be observed in muscles in experimental investigations of nutritional myopathy in pigs. In the severe form of the disease microscopic lesions of porcine muscles consist in multifocal polyphasic necrosis [3,40].

To describe a degree of histopathological changes in skeletal muscles, a semi-quantitative scale of severity of lesions was proposed: 0–normal muscles, 1–mild changes, 2–moderate changes, 3–severe changes. Mild changes were defined as the presence of individual, separated muscle fibers with increased volume and loss of striation or a very low number of degenerating muscle fibers, sometimes with mild infiltration of macrophages. Moderate changes were defined as multifocal degenerating or necrotic muscle fibers with or without infiltration of macrophages, while severe changes were defined as multifocal, relatively widespread degenerating or necrotic muscle fibers with or without infiltration of macrophages [40].

Therefore, selenium and vitamin E play an important role in prophylaxis of nutritional myopathies. With that, selenium being a constituent of selenoproteins is a key factor as it affects endocrine, immune, inflammatory and reproductive processes. Glutathione peroxidases belonging to the family of selenium-containing proteins inactivate peroxides and thereby support physiological functions of muscle tissue. Recently, it has been found that levels of selenium and vitamin E intake that earlier were considered sufficient, do not exclude the development of nutritional myopathy. In this connection, at present, the question of correspondence of existing strategies of feeding highly productive pigs in the context of assurance of their health and, consequently, quality of produced pork has been raised [40].

Conclusions

An increasing number of scientific publications show that the topicality of the problem of myopathy of slaughter animals (especially pigs) and poultry (chickens and turkey) is not diminished. Taking into consideration not only the prevalence of this disease, but also the fact that myopathy affects the most valuable parts of carcasses (*m. longissimus dorsi* and ham muscles in pigs; pectoral muscles in poultry), it leads to considerable economic losses. In this connection, a deep study of this disease is very important to reveal factors influencing its frequency and degree (category) of the development.

At present, scientific investigations are carried out in several directions with the aim of the development of prophylactic measures. For example, in the area of prevention of myopathies caused by stress, there are several studies, which were aimed at inhibition of key enzymes of muscle glycolysis after animal slaughter by peroral administration of different substances shortly before slaughter. Up to now, the results of these studies were not considered successful, despite the fact that several substances can inhibit tissue enzymes (for example, citrates and acetates –phosphofructokinase) [44]. To prevent the emergence and development of nutritional myopathies, investigations have been continued in the field of studying an effect of different doses of selenium and vitamin E with regard to sources of these substances, dura-

tion of feeding period, feed composition and other factors [1,12,40,43,45,46].

Recently, a new direction in studying myopathic conditions has emerged — nutrigenomics, which is aimed at investigation of an influence of food nutrients on gene expression and meat quality. As a measure for reducing risks of myopathy development, an addition of different adaptogens to an everyday diet is considered. With that, a choice of a diet is one of the potential tools of meat quality management influencing even muscle fiber characteristics [43,47].

Therefore, a search for nutrients-adaptogens and regulators of the targeted development of muscle tissue, that ensure its stability to unfavorable environmental factors, above all, stress factors, which have a destructive effect on muscle tissue microstructure, can be the main way of meat quality assurance with the following intensification of its production.

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Review paper

METHODS OF IDENTIFICATION OF MUSCLE TISSUE IN MEAT PRODUCTS. PREREQUISITES FOR CREATING A MULTI–LEVEL CONTROL SYSTEM

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Key words: *muscle protein, vegetable protein (soy), animal protein, BEFFE, EIA, electrophoresis, histology, identification*

Abstract

Unfair production and products that do not comply with the declared labeling are currently an acute problem in the field of technical regulation, including with regard to food safety and quality. Given the high added value and multicomponent composition, finished meat products are among the most susceptible to adulteration. Despite the best efforts of regulatory agencies to counteract these inconsistencies, the hidden substitution of cheaper or lower-grade meats is still widespread. One of the main tasks facing research laboratories and testing centers today is the detection of falsification of food products, as well as standardization and certification of techniques necessary to solve such problems. The manufacturer, aware of the current control methods, can go to the deception, using vegetable protein, new unregistered feed additives. To determine the complex changes that occur in products, it is necessary to use methodological approaches in which it is possible to reliably determine these changes. The paper presents an overview of the most commonly used methodologies for assessing the component composition of meat products. Quality assessment of meat products includes control of components of finished products. The most difficult task is to determine the proportion of muscle protein in multicomponent meat products that have undergone heat treatment.

Introduction

Since the modern technologies of the production have suffered serious changes, the problem of the multisided study of the food products, in particular the identification, is extremely actual. It is relating to initial raw and secondary materials, including the technologies for the protein products production from plant raw materials, and at the same time the introduction of artificial origin food additives into food raw materials and food products.

Same time the suddenly increased stream of various, not traditional for our market, imported products, and the increase in the production of new products at numerous small Russian enterprises on their own recipe, often allow the manufacturer to reduce the quality, and to the trade — to raise the prices.

There have been tasted, in the world practice, various methods of identification of the composition of finished meat products. However, for today there is no universal methodology, which would allow definitely to interpret the results of determining the quantitative content of muscle tissue in finished meat products.

The introduction of animal protein additives (offal, blood and its fractions, caseinates, melange, etc.) and vegetable origin (soy proteins and oilseeds) allows to stabilize the functional and technological properties of raw meat, improves the consistency, appearance and juiciness of finished meat products, while reducing their cost. Excessive application of such additives can cause falsification of products. Therefore during the assessing the quality of meat products, it is necessary to identify its composition in accordance with the declared normative document [1,2].

The main terms and definitions of the meat industry are regulated by GOST R52427–2005 [3]:

— meat product — the mass fraction of meat ingredients in its composition is more than 60%, made with or without the introduction of non-meat ingredients of vegetable and/or animal and/or mineral origin (the mass fraction of meat ingredients in the composition of canned goods for early age children is not less than 40%, in chopped semi-finished products — for baby food — not less than 45%).

— meat-containing product — the mass fraction of meat ingredients in it is from 5 to 60%, made using non-meat ingredients of vegetable and/or animal and / or mineral origin (the mass fraction of meat ingredients in the composition of canned food for early age children — from 5 to 40%, in chopped semi-finished products for baby food — from 5 to 45%).

— meat and vegetable product — with a mass fraction of meat ingredients in the composition from 30 to 60%, obtained using non-meat ingredients of vegetable origin (the mass fraction of meat ingredients in the formulation of canned food for early age children — from 18 to 40%, in chopped semi-finished products for baby food — from 18 to 45%).

— vegetable and meat product — with a mass fraction of meat ingredients in the composition from 5 to 30%, made using non-meat ingredients of vegetable origin

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(the mass fraction of meat ingredients in the formulation of canned food for early age children is from 5 to 18%, in chopped semi-finished products for baby food — from 5 to 18%).

Quality assessment of meat products includes the control of finished products components. More difficult is to determine the proportion of muscle protein in multicomponent meat products that have undergone heat treatment.

In the world practice are tested different qualitative and quantitative methods for determination of the composition of food products.

The purpose of this review is to systematize these methods and assess the prospects for their application, taking into account the creation of a multilevel control system.

Main part

Semi-quantitative methods

Microstructural (histological) analysis provides to get the information about the composition of the product as a whole and differentiate features of tissue and cell structures. It is labor-intensive and requires special equipment [4].

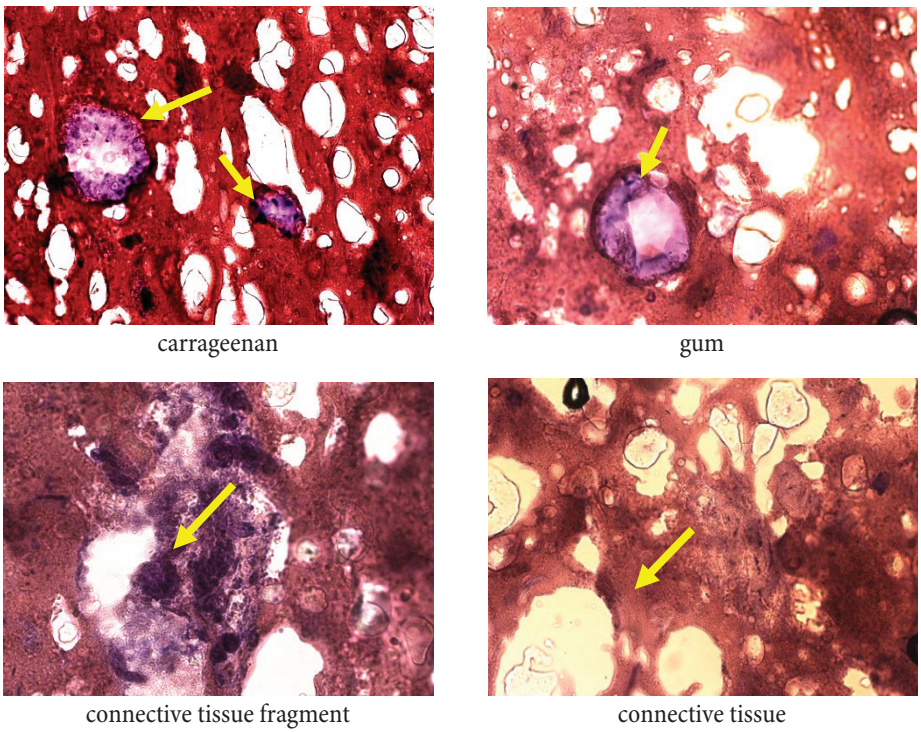
Besides the qualitative assessment of the composition and characteristics of the meat product, this method can be used for calculation of the amount of most components. Indicators can be presented in a verbal form similar as it is provided for in the German Food Legislation («often», «in sufficient quantity», «rarely», etc.), and in a strict mathematical form — in percentages indicating all necessary and sufficient statistical parameters. At the present stage of development of science and with the availability of computer image analysis systems, the widespread use of such studies has become real.

The histological method of assessing the condition and quality of raw meat, as well as the composition of finished meat products is used in many countries mainly in the course of scientific research. The data obtained using the microstructural method of research, serve as a sufficient basis for rejection of the product due to the presence of unacceptable or not provided by the formulation of components, non-compliance of the product with technological regulations [5].

The histological methods give the possibility to assess quickly and objectively the following characteristics of the product: the quality of raw materials used (freshness, cold storage effect, the degree of maturation of meat, etc.), to obtain data on the falsification of raw materials and finished products, to establish the number of unforeseen regulatory documentation additives and determine by what technological form they were used.

For example, the histological examination of Doctor's sausage, developed according to GOST [6], could contain unintended formulation carrageenan, gum, animal protein from pork skins, or a fragment of connective tissue. These inclusions detected in the sausage are shown in Figure 1.

The histological method is used in many countries for identification as composition of meat raw materials as products, mainly in the course of scientific research, and in the practice of testing laboratories, it is included quite rarely, unlike to domestic. The results obtained by histological examination serve as a sufficient basis for classifying the product adulterated, for example, by the presence of ingredients not provided for by the recipe, non-compliance with the prescription composition of the product [4,7]. At the same time, work with biomaterials isolated from food products has a certain specificity, since the matrix is subjected to research after various chemical manipulations.



carrageenan | gum | connective tissue fragment | connective tissue

Figure 1. The additives, not provided by GOST, founded in sausage [6]

The histological method is considered to be labor-intensive due to the use of special equipment and the need for certain practical skills, which causes difficulties in its application in laboratories [7].

The polymerase chain reaction (PCR) method. The DNA-diagnostic methods are used for identification of the species of animal or plant tissues in the meat raw materials and products, including those subjected to heat treatment. PCR is particularly prevalent. Establishing the species of meat using PCR is versatile- and need a specific primer (DNA fragment) for the DNA of a particular animal or plant. This method allows to detect not only species, but also genus, with a high degree of reproducibility and also allows determining 0.01% of the total volume and is characterized by the possibility of quantitative analysis [8].

The PCR method allows to detect even the single DNA of the desired species, but it can also serve as the limitation of the method: due to accidental contamination of the sample in trace amounts, there is a high probability of issuing a false positive result [9].

The example of identification shown in Figure 2. Currently it is used GOST 31719–2012 «Food and Feed». Express method for determining the raw material composition (molecular)» [10] on the PCR method, which establishes the determination of the species of meat and vegetable ingredients contained in feed, food, food raw materials of plant, animal origin, including those subjected to heat treatment.

Electrophoretic methods: types and application principles. The method of electrophoresis separation of proteins in gel — is widely used in the study of proteins. Electrophoresis consists in the separation of the protein mixture by mass (1DE), and two-dimensional (2DE — two dimensional electrophoresis) — in the sequential use of two properties of proteins: charge and mass, which is necessary for maximum separation of the protein mixture.

The method of electrophoretic determination of the composition of finished products is based on thermal denaturation and extraction of proteins from minced meat, followed by separation of extracted protein fractions in polyacrylamide gel. To obtain more complete picture of the protein composition in the last decade began actively apply the method of two-dimensional electrophoresis. It is established that, closely located bands in the gel can be superimposed. This property prevents the determination of a large number of proteins by one-dimensional electrophoresis. The method of two-dimensional gel electrophoresis in polyacriamide gel (PAAG), combining two different separation procedures, allows the identification of several hundred and sometimes thousands of proteins and peptides. The results are obtained in the form of a protein map in a two-dimensional coordinate system: on the OX axis — the are located the isoelectric point of proteins, on the OY axis — their molecular masses.

The main task of this method is the maximum extraction of proteins from samples that are solubilized with lyzing solutions. Then, dissociated polypeptide chains are separated by isoelectric focusing (IEF) [11]. IEF is the movement of proteins in the pH gradient under the action of an electric field to the pH region equal to the isoelectric point (IET) of the protein molecule [12]. The effect of protein IEF has long been known, but attempts to apply it to protein fractionation have long been unsuccessful due to the difficulty of creating a pH gradient. This problem was solved with the development of synthetic ampholyte-carriers. The ampholites, specially synthesized amphoteric compounds, are polyamine-polycarboxylic acids, which are produced by different companies under different names: ampholines, pharmalites, servalites [13].

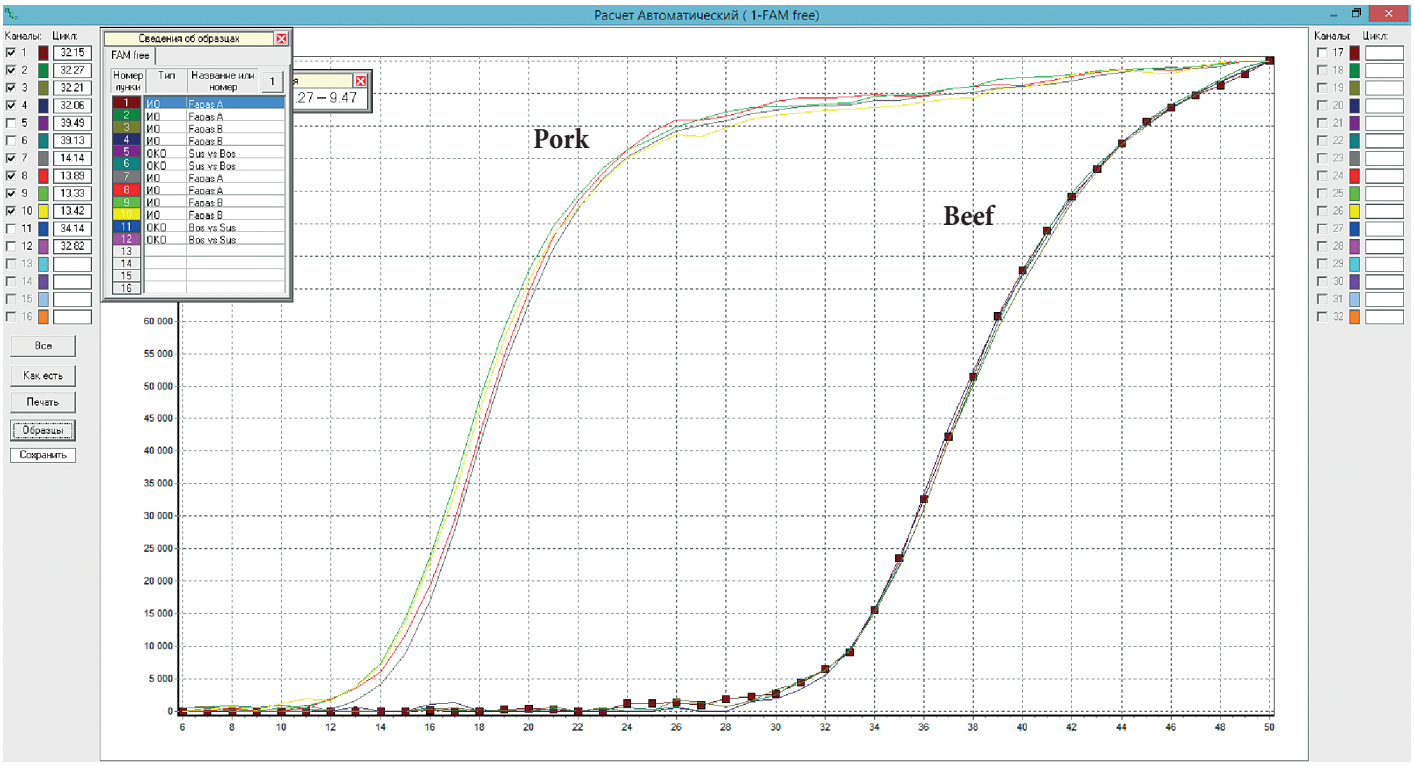


Figure 2. DNA identification of meat by PCR, in boiled sausage

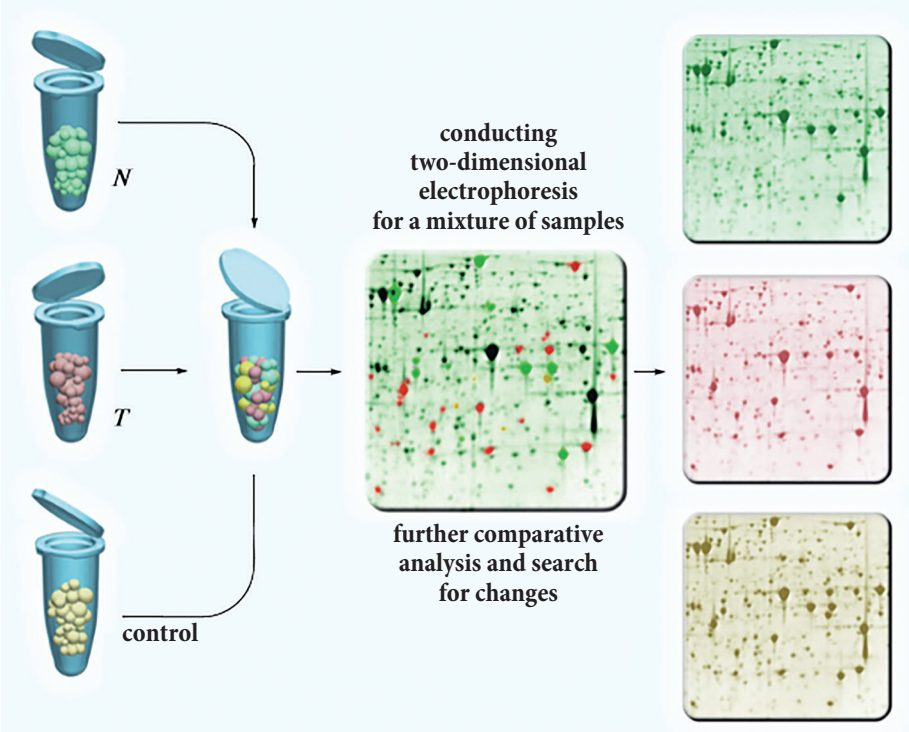


Figure 3. Results of electrophoretic fractionation (2D with cyanine label) of muscle tissue proteins [14]

Thus, in Figure 3 presents 2D electrophoregrams of muscle tissue protein fractionation with cyanine label. Protein extracts of tissue samples are labeled with two different cyanine colorants (N, T) having different wavelengths of emitted radiation, and mixed. Next, 2D is carried out, then for each fraction the fluorescence intensity is measured and the difference between the two labels is determined. The method allows to assess their quantitative content by the ratio of the intensity of fluorescence spots also.

There was an improved technology 2D-DIGE — «three-dye method», which provides for the use of a special» internal standard», created by combining equal quantities («pooling aliquots») of both test samples [15]. The test samples (experience and control) are labeled with colorants, and the combined internal standard is labeled with a third colorant. Next, the analyzed samples are mixed, and the proteins are fractionated 2D. It is believed that the use of such internal standard allows to reduce the variability of 2D-DIGE gels, facilitates the use of image analysis software (for example, Sangene, DeCyder), with the help of such technique it is possible accurately to determine the amount of protein with a certain statistical certainty [16].

Since the beginning of the postgenomic period, so-called «non-gel» strategies have been developed and actively applied as an alternative to the traditional proteomic strategy [17]. Typically, these strategies include specific sample preparation in which the complex protein mixture is subjected to trypsinolysis or cynogen bromide hydrolysis. The resulting peptide mixture is then fractionated by capillary chromatography in one or more steps in the so-called multidimensional liquid chromatography (MDLC) [18].

The serious disadvantage of 2D is that the analysis is limited only to a certain subgroup of the cell pro-tein population [19]. This is partly due to the chemical properties of 2D-based systems, which distinguish mainly between basic and hydrophobic proteins and thus exclude analysis of most receptors and trans-membrane proteins [20]. However, the strongest limitation of 2D assays is due to the limited dynamic range, which covers only 2–3 orders of amplitude (Pico-nanomolar range), while the ranges of cell protein expression in most tissues cover more than eight orders of amplitude (micro — and femtomolar range). Thus, 2D analysis of non-fractionated tissue samples is limited by the presence of high molecular weight proteins. In this regard, a wide range of methods for preliminary fractionation of complex samples has been developed to solve this problem [21]. Currently, rapidly developing methods of comparative prote-omics and mass spectrometry (MS) identification have to some extent replaced the classical studies based on 2D electrophoresis.

However, it is considered to be quite time-consuming and costly due to the fact that mass spectrometry MALDI (MS/MS) is used for quantitative analysis of gels [22].

Calculation methods

According to the recipe (tab). The aim of the method is to calculate the amount of muscle tissue of the finished product in accordance with the recipe. Standards for manufactured products describe the General definitions and estimates of the feedstock.

The document, that allows to calculate the amount of muscle tissue on the tab of cooked sausages -according to the method described in source [5]. The application of the method implies absolute honesty of the product manufacturer. The high proportion of the falsification cases of the cooked sausages composition (up to 90% of their volume

produced according to GOST) leaves no hope for the correct calculation.

BEFFE. An indicator that allows to calculate the amount of muscle tissue, for example, in Germany is the BEFFE (bindegewebseiweißfreies Fleischeiweiß — meat proteins, that do not contain connective tissue).

The importance of BEFFE is defined by the German Food Commission in the document «Guidelines for meat and meat products of Germany» as «the difference between total protein and the sum of foreign proteins, foreign non-protein nitrogen compounds and connective tissue protein». Analyzing the methods and requirements for finished meat products in the EU (on the example of the German experience), the standards describing the General definitions and estimates of raw materials and requirements for designations, beef, pork and poultry meat is divided into three groups or categories. For example, beef with I category per 100 g in product should contain protein — 18.6 g, fat — 16 g.

The amount of muscle tissue (meat) in the finished product can be determined by calculation based on the results of a complete analysis of the content of chemical components (total protein, fat, moisture, ash, unbound water) in the product. Water, wich content is four times more than amount of protein, is rated as unbound water. Cooked sausages typically contain up to 15% unbound water.

According to the standards, the value of BEFFE abs should be not less than 7.5%, and BEFFE real in meat — not less than 75%.

General calculation formula for this indicator:

$$\text{BEFFE abs} = \%\text{Pr-Coll/Pr}\%$$

$$\text{BEFFE real} = (\text{BEFFE abs}*100)/\%\text{Pr},$$

where %Pr, Coll/Pr% — the amount of total and collagen protein, g/100 g, respectively.

In accordance with the standards of German food legislation, meat products of the highest quality should be differ from ordinary products by a special selection of raw materials [23].

The disadvantage of the method is the need for a long study of the chemical composition of various objects (pork, beef, poultry, etc.) and the preparation of normative documents. There are limitations in this method, consisting in multiple studies of the chemical composition of raw meat and legislative consolidation of certain norms. According to the experience of German colleagues, it is proved, that in order to obtain statistical data, it was necessary to study at least 100 samples of each species, which from an economic point of view is not always advisable.

Quantitative method

Determination of animal protein by the ratio of amino acids. In Russia there was developed a method for calculating the amount of animal protein by the connective tissue, that is relative to the content of the oxyproline aminoacid [24]. However, it does not allow full identify the composition of the finished wrought products. In German laboratory practice, there is a more accurate method for determining the content of hydrolyzed protein or connective tissue protein (collagen). It is based on sequential extraction with further application of photometric method to identify hydrolysis products (4-dimethylaminobenzaldehyde) and calculation.

In our country, in order to find out the nutritional value of meat, use the ratio of two amino acids: tryptophan and oxyproline. The ratio of tryptophan to oxyproline (4: 1) is inversely related to the content of connective tissue.

The proteins of myogen and myosin may be of interest in the aspect of the case in point. It is proved, that muscle sarcoplasm contains myogen, which amounts 20 … 30% of all muscle tissue proteins, it cannot be attributed to typical globulins, it is easily extracted by the water. The ratio of histi-dine: arginine: lysine in the myogen is 2:4:6.

The composition of myosin is dominated glutamic and aspartic acids, leucine, lysine and arginine. The half of myosin molecule is constructed from all these five aminoacids; the ratio of histidine: arginine: lysine — 2:7:12 [25].

Methods of Mass-spectrometry. The identification of muscle tissue biomarkers. The selection of biomarkers of various components is the promising area of research in the field of determining the composition of finished meat products. Mass spectrometry is an important technique for protein characterization and sequencing. The two main methods of whole protein ionization are electrospray ionization (ESI) and matrix laser desorption/ionization (MALDI). According to the characteristics and mass range of available mass spectrometers, two approaches are used to characterize the proteins. In the first case, intact proteins are ionized by any of the two methods described above, and then injected into the mass analyzer. This approach is called the «top-down» protein analysis strategy. The «top-down» approach, however, is largely limited to low-throughput studies of a single protein. In the second case, the proteins are enzymatically digested to smaller peptides using proteases such as trypsin or pepsin, either in solution or in gel after electrophoretic separation. Other proteolytic agents are also used. Collected peptide products are often separated by chromatography before being introduced into the mass analyzer.

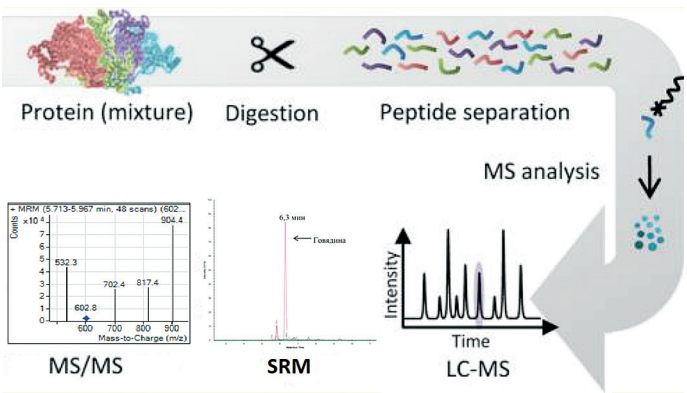


Figure 4. General scheme of preparation and determination of peptide markers [26]

Immunoanalysis methods

Immunoanalytic methods are based on highly specific recognition of antibodies of certain structures in antigen molecules. When characterizing the composition of meat products, protein antigens are usually considered, for which the recognizable site (epitope) is from 5–7 to 15–20 aminoacids. The main question during the using of immunoanalysis — is the choice of epitope, peculiar to a strictly defined species. For example, by selecting the antibody, which recognizes a specific protein in the muscles of a pig, we must be sure, that the muscles of a chicken or cow will not detect a protein, that binds to this antibody (just as effectively, or worse, but enough to produce a non-specific signal). Therefore, immunoanalysis distinguishes well systematically distant from each other organisms or non-muscular additives in meat products, but if necessary, to distinguish closely related species commercially available specific antibodies may be absent, and the development of the analysis will have to start with the search for a unique antigen and antibodies to it.

Despite to these limitations, today there are a number of successful and implemented in practice development of immunomethods for the control of meat products. Almost all of them refer to either microplate enzyme immunoassay (EIA) or immunochromatographic analysis (ICA).

EIA, in the microplate version, includes several stages: preliminary immobilization by antibodies or antigen on the surface of the wells of polystyrene microplate; introduction of a sample containing analyte into the wells; introduction of the enzyme-labeled immunoreagent; preparation of a colored product from the substrate during an enzymatic reaction. The using of the enzyme as a label can significantly increase the analytical signal, since a single enzyme molecule can catalyze the conversion of a large number of substrate molecules into a product. The stages of immunochemical interactions are separated by washing the wells of the microplate with a buffer containing a detergent to remove reagents that have not reacted.

For the determination of low molecular weight compounds, as a rule, a competitive EIA format is used (Figure 5A). High-molecular antigens due to the presence of several disjoint binding sites (antigenic determinants) on surface of them can be detected using not only competitive, but also usually more sensitive «sandwich» format of analysis (Figure 5B). In a situation where the target protein during processing of raw meat can be fragmented and partially denatured (under the action of proteases and temperatures), competitive methods of analysis may be more informative. Effectively working systems realizing both competitive, and «sandwich»-format of EIA are described.

Enzyme immunoanalyses are provided with relatively inexpensive serial equipment that automates the stages of reagent introduction, incubation, washing and final measurements. In poorly equipped laboratories, the analysis can be carried out manually and requires only the use of a vertical photometer for optical measurements in the wells of the microplate. The EIA is a quantitative method that allows to calculate the concentration of antigen in the sample and the proportion of the corresponding raw material in the tested product on the basis of the results obtained.

The EIA is effective for controlling the presence of prohibited types of meat in products, which is of fundamental importance for consumers whose national or religious views do not allow the consumption of certain species of animals meat [32]. It allows to determine both individual types of meat raw materials and the total content of meat raw materials from different sources [33, 34,35,36], as well as to identify additives of non-meat origin in the product [37]. The EIA method for the quantitative determination of soy protein in the composition of various meat, meat-containing and meat-vegetable food products has been developed in Russia [38].

The microplate EIA allows simultaneous testing up to 40–80 samples. The duration of the analysis is usually from 1.5 to 3 hours. The possibility of the EIA in the kinetic mode with a reduction in duration to 30–40 minutes is shown, but these approaches are poorly developed methodically and are often accompanied by a decrease in the accuracy of quantitative measurements.

For express monitoring, immunochromatography is of the greatest interest, in which all specific reactions occur on a test strip with deposited immunoreagents. The test strip is a multimembrane composite, in certain areas of which all the necessary immunoreagents and their complexes with a label are previously immobilized (Figure 6). The most frequent used label in ICA is a gold nanoparticles. Upon contact of the test strip with a liquid sample under the action of capillary forces, the movement of the sample components along the membrane occurs, which is accompanied by immunochemical reactions with reagents applied to the membrane. These interactions lead to the formation of colored immune complexes in certain areas of the test strip. Based on the presence or absence of staining of the test line of the test strip, a qualitative conclusion is made about the presence in the sample of a controlled compound — analyte — or about its exceeding the limit concentration (the control line is colored regardless of the composition of the sample and is used to confirm the effectiveness of the reagents.) Quantitative determination of analyte content is carried out according to the intensity of staining, fluorescence or other characteristics of the associated marker, using additional equipment (scanner, video recorder, smartphone camera, etc.). The ICA, like the EIA, can be implemented in competitive and «sandwich» formats.

The advantages of ICA are ease and speed of realization (10–15 min), visual (non-selective) registration of results, and, consequently, possibility of carrying out the analysis on a place of sampling. As a rule, the ICA is inferior to other immunomethods in sensitivity, but in relation to the composition of meat products, this factor is not limiting, and if necessary, additional reagents can be included in the composition of the test-strip, providing signal amplification

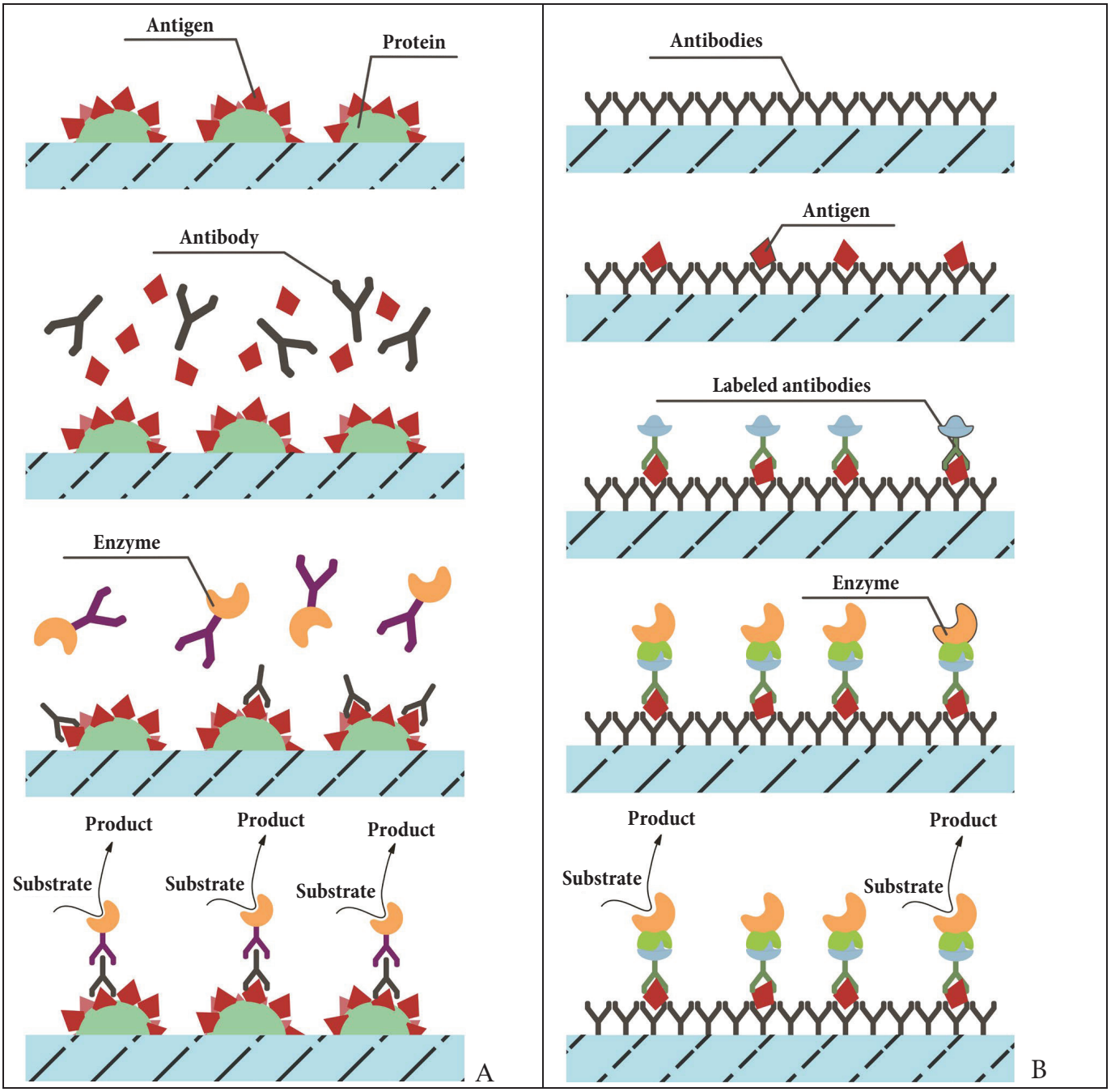


Figure 5. Scheme competitive format (A) and «sandwich»-format (B) of EIA

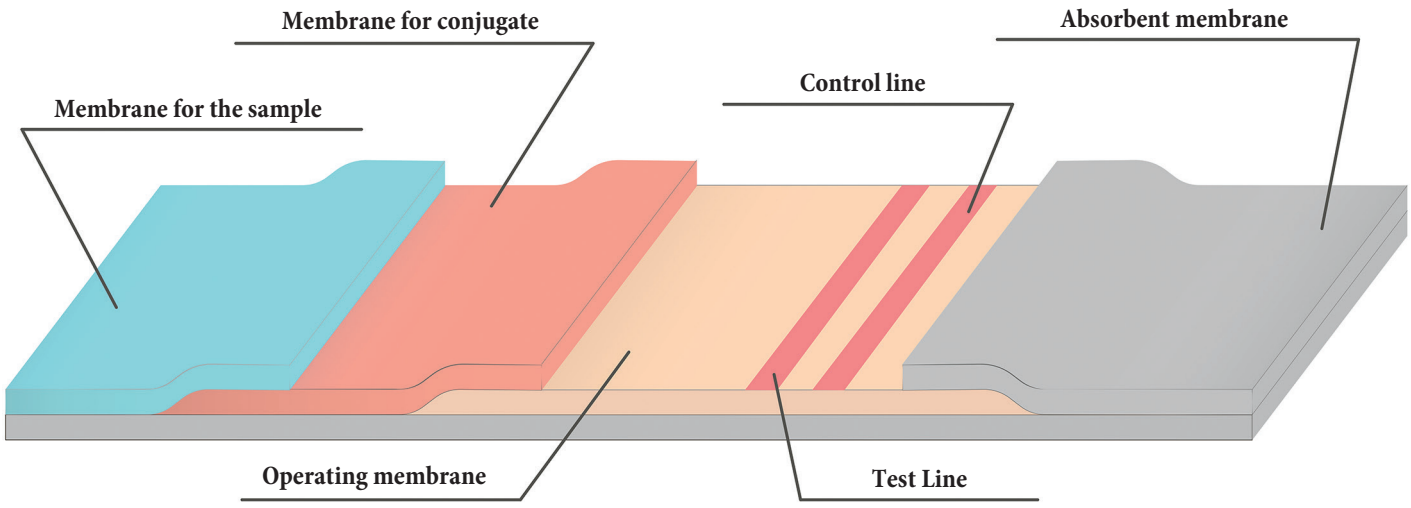


Figure 6. Scheme of immunochromatographic test-strips

and sensitivity increase by one to three orders of magnitude with preservation of expression and methodological simplicity of testing. Despite the existence of a number of developments on quantitative assessment of analyte content using the ICA, the dominant practice for today, is the use of it for quality control: detection of prohibited components in manufactured products [39,40].

Conclusion

In the world practice, various methods of identification of the composition of raw materials and ready meat products have been tested. However, at present time, there is no universal methodology that would allow to interpret unambiguously the results of determining the quantity of muscle tissue in finished meat products and the use of undeclared components used in meat production.

Existing standardized methodologies do not allow to decompose fully the composition of the food product into its constituent components. The increasing demands of consumers and the emerging needs of food manufacturers encourage the development and implementation of effective control methodologies, in which a special place is given to the control of the composition of food.

In this regard, we found it relevant to form a set of methods of screening and arbitration control of meat products in order to create a multi-level control system aimed at identifying violations of established formulations.

Applied proteomics and immunodetection aimed at finding the biomarkers of the composition of objects of plant and animal origin and identification signs of authenticity of products will allow quickly and with a high level of reliability to detect cases of falsification.

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