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Effect of preparation of the test samples by dissolution on the indicators of the protein composition of animal meat

Elena V. Tsaregorodtseva*[1], Tatyana V. Kabanova[1], Maria V. Dolgorukova[1], Elena L. Matveeva[2], Marina S. Gugkaeva[3], Albina K. Kornaeva[3], Natalia A. Shkaeva[4], Irina A. Lykasova[5]

[1]Mari State University, Lenin Square 1, Yoshkar-Ola City- 424000, Russia
[2]Kazan Innovative University named after V.G. Timiryasov, Moskovskaya Street 42, Kazan City-420111, Russia
[3]Gorsky State Agrarian University, Kirova Street 37, Vladikavkaz City- 362040, Russia
[4]South Ural State University, Lenin Prospect 76, Chelyabinsk City- 454080, Russia
[5]South Ural State Agrarian University, Gagarin Street 13, Troitsk City- 457100, Russia

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ABSTRACT

The article presents the research of the protein composition of meat of wild animals (elk and wild boar) and domestic animals (beef), by their isolation and separation methods due to their different solubility in different solutions, conducted in the laboratory of Biological Safety of Food Systems of the Department of Meat and Dairy Products Technology of Mari State University. The total proteins or protein fractions were extracted from a homogenized chilled meat sample. Then, the colorless proteins in the extract were transferred to the colored product, adding a biuret reagent, and studied the D optical density of separate protein fractions on the photoelectric concentration colorimeter PCC-2, determining the absorption of emitting at a wavelength of 540 nm. The protein content of the samples was expressed in the mass fraction of the protein fraction in the sample in g/ 100g of meat and in percentages. The calculation was based on the previously found linear calibration dependence of the optical density on the concentration of the standard protein. Differentiation of proteins by solubility (water-soluble, soluble in salts, alkaline-soluble, albumins, globulins), fractional composition (sarcoplasmic, myofibrillar and connective tissue), peculiarities of the biochemical structure (high-grade and inferior). Protein extracts of muscle tissue of wild and domestic animals obtained during sequential extraction are used to quantify the protein content of a certain fraction, as well as to research the properties of proteins.

*Corresponding Author

Name: Elena V. Tsaregorodtseva
Phone: 89033146175
Email: Smolentsev82@mail.ru

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INTRODUCTION

From the muscle tissue of meat, proteins can be isolated and divided due to their different solubility in different solutions. Soluble proteins are mainly found in the sarcoplasm of muscle fibers, and soluble salts form myofibrils (Yakobson *et al.*, 1998). Insoluble protein fractions in water-salt solutions are conditionally called stroma proteins, which contain proteins of sarcolemma, nuclei and intracellular connective tissue proteins (Schetters *et al.*, 1990). Muscular proteins, according to their solubility, are divided into three groups - myofibrillar (55% of

total proteins), sarcoplasmic (35% of total proteins) and connective tissue proteins (3-5% of total proteins) (Jaworski *et al.*, 2016).

Sarcoplasmic proteins: myogen, globulin X, myoalbumin, myoglobin - are located inside the membrane of muscle cells in the sarcoplasm and account for about 30-35% of the total number of proteins in the muscle (Mammerickx *et al.*, 1981). These proteins are soluble in water or in solutions with a low ionic power (<0.6 millimole / cdm). Proteins of sarcoplasm are water soluble, mostly high-grade and well digested. This group of proteins also includes oxidative enzymes, heme pigments, glycolytic enzymes, which are responsible for glycolysis, and liposomal enzymes (Ungar-Waron *et al.*, 1992, 1999; Konishi *et al.*, 2018).

Myofibrillar proteins, or salt-soluble proteins, are insoluble in water, but most of them are soluble in a solution of table salt with a concentration of more than 1% (Ruggiero and Bartlett, 2019). This group consists of approximately 20 individual proteins which are part of the myofibrils of the contractile muscle. Myofibrillar proteins include the contractile proteins myosin, actin and actomyosin, as well as regulatory proteins tropomyosin, troponin and alpha and beta actin. Myofibrillar proteins provide contractile muscle function.

Connective tissue proteins of meat form a net that envelops the muscle fibers and pervades the entire muscle. Because of this, they serve as a frame that supports the structure of the muscle. These include proteins of sarcolemma and intramuscular connective tissue (collagen, elastin, reticulin, mucins, mucoids, lipoproteins, acidic and residual proteins). These proteins, except for collagen and elastin, can be extracted from muscle tissue with a 0.25% solution of NaOH (Kono *et al.*, 1982; Khudhair *et al.*, 2016).

Albumins of meat are soluble in water and precipitate only at more than 50% saturation of the solution with salt. Albumin fraction is made up of proteins of sarcoplasm: myogen, myoalbumin and myoglobin, which are extracted with water. Globulins are soluble only in solutions of salts of medium concentration (8-15%). In solutions with a higher and lower salt concentration, the solubility of globulins decreases. The globulin fraction is made up of myofibril proteins: myosin and actomyosin. When the protein extract is divided by the dialysis method, the protein precipitate from the globulin fraction first acquires an amorphous form, then gradually begins to crystallize and acquires their specific crystal form.

MATERIALS AND METHODS

The total proteins or protein fractions were extracted from a homogenized chilled meat sample. Then, the colorless proteins in the extract were transferred to the colored product, adding a biuret reagent, and studed the D optical density of separate protein fractions on the photoelectric concentration colorimeter PCC-2, determining the absorption of emitting at a wavelength of 540 nm. The protein content of the samples was expressed in the mass fraction of the protein fraction in the sample in g/ 100g of meat and in percentages. The calculation was based on the previously found linear calibration dependence of the optical density on the concentration of the standard protein.

Albumins and globulins of muscle tissue are the most common proteins. Usually they are found together, and their separation from each other is based on their different solubility in water and the varying degree of salting out by mineral salts.

To clean solutions of meat proteins of different kinds from salts (desalting) and low-molecular substances, dialysis method was used. The method of dialysis is based on the inability of large protein particles to permeate the pores of the semipermeable membrane (cellophane), while other molecules and ions easily pass through it. 10 ml of salt extract filtrate from meat of various kinds were poured into the dialyzer and the pouch was immersed in a 1 liter glass with distilled water. Water in a glass was changed every 5-10 minutes, testing the reaction to chloride ions. Dialysis continued for 1.52 hours until the chloride ions test became very weak and precipitation did not stop when silver nitrate was added to the water sample. This indicated the completion of dialysis, that is, the almost complete diffusion of salt from the dialyzer into an external solvent. In the pouch at this time, the previously transparent solution becomes muddy, due to the precipitation of globulins insoluble in distilled water. The pouch was taken out of the glass and the contents were filtered through a paper filter. On the filter there are globulins, water-soluble albumins remain in the filtrate.

The water-soluble proteins extracted from the tissues were divided into separate fractions using a precipitant with the salting out method. For this purpose, ammonium sulfate was added to the protein solution, gradually increasing the electrolyte concentration at a certain pH and temperature. Under such conditions, the albumins precipitate, which is filtered through a dry paper filter.

It was found and proved the presence of proteins with the help of color reactions to proteins (biuret

reaction). 1 ml of the filtrate and 4 ml of the biuret reagent were mixed and held for 30 minutes and the optical density of the protein fraction was measured on a PCC-2 photoelectric concentration colorimeter at a wavelength of 540 nm (green light filter) in 1 cm thick cuvettes.

Further, when calculating the concentration of albumins and globulins, the optical density of the extracts prepared from muscle tissue was substituted into the single-factor regression equation by a linear relationship: D = 0.079 + 0.38C and the protein concentration in the muscle extract was calculated (C, mg/ ml). For the convenience of characterization of protein complexes of meat obtained from different species of animals, the conversion of absolute units to relative ones was performed. Methods of mathematical statistics were used to process the results.

RESULTS AND DISCUSSION

Analysis of the fractional composition of meat proteins obtained from domestic and wild animals showed that the total weight of protein in beef is 19.88 ± 4.77 g / 100 g of meat, which is less than in meat obtained from wild animals, including elk meat by 2.44 g / 100 g meat (P P ≤0.05) and wild boar by 1.35 g / 100 g of meat (P≤0.05). There is no significant difference in the total protein mass fraction in the meat of elk and wild boar using photometry (Table 1).

Thus, meat of wild animals contains an increased content of proteins compared to meat of domestic animals, which is associated with the natural habitat and the possibility of building muscle mass due to a lively way of life

The content of the water-soluble protein fraction is the greatest in meat of the boar 7.69 ± 0.35 g/100 g of meat (P ≤0.01), and of elk meat 7.63 ± 0.58 g/100 g of meat (P ≤ 0.05) compared to beef. This is explained by the presence of myoglobin protein, which causes a characteristic red color of muscle tissue, the most intense in meat of wild animals, as well as a large amount of sarcoplasm easily separating during pressing. This is confirmed by the water-binding capacity data, which was 23.1% in beef, 60.15% in the elk meat and 68.38% in the boar meat. High humidity provides maturation, softening and flavoring of meat, but it contributes to more intensive development of pathogenic microflora. Consequently, when controlling the safety of meat of wild animals, a qualitative analysis of the microbial contamination indices should be carried out.

The mass fraction of soluble in salt proteins in beef

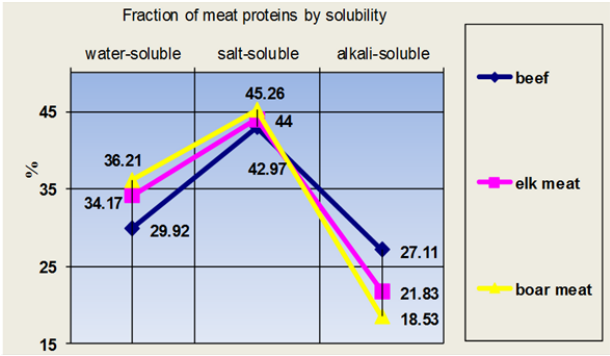

Figure 1: Fractional composition of protein, % to total protein

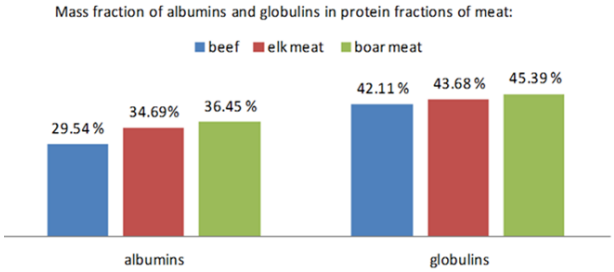

Figure 2: Protein fractions of albumins and globulins, % of total protein

was 8.54 ± 0.41 g/100 g of meat, which is less than in meat of elk by 1.28 g (P ≤ 0.05) and boar meat by 1.07 g/100 g of meat (P ≤ 0.05), indicating a significantly higher presence of full-fledged myofibrillar proteins in meat of wild animals, which during digestion in the gastrointestinal tract will be almost completely used by the human body for anabolic purposes and the formation of their own muscle mass.

Meat of wild boar contains a minimum amount of alkali-soluble proteins 3.93 ± 0.26 g / 100 g of meat, and beef is the largest (P ≤ 0.05). This is due to the age of the animal. According to the content of connective tissue proteins between beef and elk meat, there is no reliable difference, therefore, the content of collagen fibers in the muscle tissue of the longest muscle of the back is the same in the meat of these species.

The fractional composition of the proteins of the meat of wild animals is of undoubted interest for characterizing its protein complex. The graph of the percentage content of different protein fractions in % of the total protein in different types of meat makes it possible to judge the ratio of high-grade and inferior proteins in meat of wild animals and beef (Figure 1).

The prevalent fraction of proteins in beef, meat of elk and wild boar are soluble in salt proteins (Table 1). Their content is at the level of 42.97-45.26% and sig-

Table 1: Fractional composition of meat proteins, g / 100g of meat

Fractions of meat proteins by solubility	Beef	Meat of elk	Meat of wild boar
The total weight of the protein, including by fractions:	19.88 ± 0.64	22.32 ± 0.58*	21.23 ± 0.18*
Water fraction	5.95 ± 0.50	7.63 ± 0.58*	7.69 ± 0.35**
Salt fraction	8.54 ± 0.41	9.82 ± 0.27*	9.61 ± 0.23*
Alkaline fraction	5.39 ± 0.49	4.87 ± 0.40	3.93 ± 0.26*

Here and below: *- P≤0.05; ** - P≤0.01

nificantly more in meat of wild animals in comparison with beef (P≤0.05).

The sarcoplasmic protein myoglobin provides a darker color of the meat of wild animals so the percentage of sarcoplasmic proteins in the meat is larger and corresponds to 36.21-34.17% versus 29.92% in beef.

The proportion of alkali-soluble proteins in beef accounts for 27.11% of the total protein, which is higher than in wild-animal meat by 5.28- 8.58%. From the point of view of modern nutrition science, the content of a certain proportion of connective tissue proteins - collagen and elastin, does not reduce the biological and nutritional value of the product, but the assimilation of collagen is within 0.85 because of its inferiority. Thus, unlike beef in meat of wild animals, the ratio of high-grade proteins to inferior ones is higher, which characterizes elk and boar meat as biologically high-grade protein meat raw materials. The ratio of the albumin and globulin fractions confirms this position (Figure 2).

The maximum protein content of albumin fraction is fixed in meat of wild animals in comparison with beef. So out of 100 g of muscular elk tissue, 7.72 g of albumins were isolated, and of the boar was 7.70 g / 100 g of the test sample. This amounts to 34.69 ± 2.81 and 36.45 ± 2.78% of the total protein mass in the samples, what is certainly is significantly more than in beef (P≤0.05). This is explained by the presence of a larger mass fraction of the myoglobin protein in the albumin fraction of meat of wild animals, which causes a rich red and dark red color of meat and proteins of albumins and globulins X, which form the basis of sarcoplasm. So the meat of wild boar and elk with organoleptic evaluation and determination of water binding capacity turned out to be more humid, due to greater watering of muscle cells, accordingly it has a higher albumen content compared to beef, by about 0.5 unit more.

The share of the globulin fraction in the meat of wild animals is slightly higher than in beef and amounts to 9.73 g / 100 g in elk meat or 42.11 ± 3.51%, in boar meat 9.59 g / 100 g or 45.39 ± 3.14% of the total protein. This is explained by a lively lifestyle, a well-developed muscle mass and as a consequence of a larger proportion of contractile proteins myosin and actin, in the structure of meat.

CONCLUSION

The muscle protein extracts resulting from the use of different solvents are a mixture of proteins. Using sequential extraction of muscle proteins with water, saline and alkaline solutions, various protein fractions were obtained. Proteins of myofibrils are extracted from meat much more difficultly than proteins of sarcoplasm, due to complexation between proteins, and also between proteins and other components of myofibrils. Solutions of salts were used to dissolve them. Proteins of the sarcolemma and intramuscular connective tissue collagen, elastin, reticulin, mucins, mucoids, lipoproteins, acid and residual proteins, were extracted from the muscle tissue by a 10% solution of NaOH. The results of protein separation by dialysis and salting out methods, based on the ability of albumins and globulins to precipitate at different concentrations of salts in solution, confirmed the position that the albumin fraction is made up of proteins of sarcoplasm, and the globulin fraction is the high-grade myofibrillar proteins of meat. In the meat of wild animals a significantly higher proportion of high-grade sarcoplasmic and myofibrillar proteins was established and less connective tissue than beef. The quantitative ratio of different fractions of proteins, their state determine the technological properties of raw materials and products, their biological value.

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