ON THE PROBLEM OF INVESTIGATION OF MUSCLES CYTOSKELETAL PROTEINS BY SDS-ELECTROPHORESIS IN POLYACRILAMIDE GEL

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Proteins of muscle cytoskeleton, as discovered in 60-s of our century, have not been well studied by the present time, and their role in a muscle architectonics and functions is not clear. Nevertheless, about 20 % of total protein content of the muscles account for these proteins, they are large molecular proteins (thus, titin has a molecular mass of 2800 kDa), and it can be supposed that these proteins have an important role in forming toughness of meat.

The investigation of cytoskeleton proteins in native state has many difficulties, caused by their morphological position and closeness by their content and structure to the main muscle proteins and collagen of muscular connective tissue. Therefore, the prevailing method of their investigation is the method of electrophoresis in polyacrilamide gel (PAGE).

In spite of the fact that main proteins of cytoskeleton - connectin and nebulin - have been an object of intensive investigations during two latest decades, there are a number of technical difficulties that need to be overcome.

In the present work we wanted to describe some improvements as suggested by us in preparation and carrying out electrophoretic investigations of cytoskeletons proteins. It concerns the method of solubilization of connectin from the muscle mass and use of a gradient polyacrilamide gel. Some results on the state of cytoskeleton proteins of salted and unsalted meat, obtained with the use of the offered technique are also described.

The method of solubilization of connectin as offered by us is based on a number of principles described in the works of K.Wang (1982) and R.H.Locker et al (1984); however the scheme of solubilization was changed by us.

About o,5 g of muscular tissue was homogenized in cold in 5 ml of 0,5 mM of aqueous solution of EDTA, containing 0,5 mM of proteases inhibitor - phenylmethylsulfonilfluoride. After clarification by centrifugation the homogenate was used as a source of a connectin. The solution, used for solubilization, was as follows : 25 mM Tris-buffer, 75 mM of glycine, 2 mM EDTA, 2 mM dithiotreitole, 2 % of sodium dodecylsulfate; pH=8,8. Equal volumes of muscle homogenate and heated to 50 °C solubilizing solution were mixed, then the mixture was heated at 50 °C during 30 min.

Then, during preparation of the mixture of solubilized proteins for electrophoresis we used the original technique described in the work of J.D. Fritz et al (1989).

The SDS-electrophoresis was carried out according to a new scheme with the use of a system of a gradient polyacrylamide gel with the interval of porosity of 4-10 %. The porosity of a condensing gel was 2,5 %. Electrode buffer with pH = 8,8, containing 10 mM of 2-mercaptoethanol was used for the optimization of separation of protein bands.

M. longissimus dorsi of thawed beef N and PSE (pH=6,0 and 5,7, respectively) was used as the object of investigations.

Time of autolisys of beef before freezing was 48 hours. The beef was cured with a cure containing 2,2 % NaCl and 0,3 % of polyphosphate "Polyphan - AE" ("Reatex", Moscow). The duration of curing was 24 hours.

Results and discussion

Data about the contents of connectin and nebulin in 0,01 mg of protein of investigated samples as calculated from obtained electrophoretograms in PAGE, are presented in the Table.

Data of electrophoretic (in PAGE) analysis of beef of two quality groups (N and PSE)

No.	Protein	Amount of applied protein (mg)	Amount of cytoskeletal proteins on electrophoretogram (rel. units)	Amount of cytoskeletal proteins as per 0.01 mg of added protein (rel. units)	Total amount of connectin per 0.01 mg of applied protein (rel. units)
			Beef N		
1.	Connectin α	0,009	3,4	3,8	6,8
2.	Connectin β	-"-	2,7	3,0	
3.	Polypeptide with M.m. 1000 kDa	-"-	1,7	1,9	
4.	Nebulin	-"-	2,9	3,2	
			Beef PSE		
1.	Connectin α	0,009	1,46	1,6	4,3
2.	Connectin β	-"-	2,47	2,7	
3.	Polypeptide with M.m. 1000 kDa	-"-	1,04	1,2	
4.	Nebulin	-"-	2,0	2,5	
			Cured beef PSE		
1.	Connectin α	0,007	1,4	2,0	5,0
2.	Connectin β	-"-	2,1	3,0	
3.	Polypeptide with M.m. 1000 kDa	-"-	-	-	
4.	Nebulin	-"-	-	-	

The data show that:

1. Both forms of connectin (α and β) are present in all the samples (cured and uncured); polypeptide with molecular mass 1000 kDa, which splits off from connectin α with the formation of connectin β is also identified (as indicated in many investigations, King N.D. et al, 1981, Kim K. et al, 1992).
2. PSE beef has an essential decrease in the content of connectin (α and β) and nebulin as compared to normal beef. There are indications in literature that the activitiy of own proteinases of meat is directed primarily to proteins of the cytoskeleton (Owen A. et al, 1980, King N.D. et al, 1981). In the study (Borissova M.A. et al, 1995) it is shown that activity of endogenous proteinases of PSE meat is significantly higher, that of N meat. One can suppose that a decrease in the content of connectin and nebulin of PSE meat as compared to N meat is caused by this factor.
3. In the electrophoretograms of cured PSE beef a band of nebulin and polypeptide with molecular mass 1000 kDa disappears completely. It is probably that under the influence of common salt and phosphate a degradation of these proteins into low-molecular components occurs. Therefore, common salt and phosphates not only cause swelling and partial destruction of main myofibrillar proteins - myosin and actin, but also contribute to degradation of main proteins of muscular cytoskeleton, increasing tenderness of meat.

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