THE INFLUENCE OF AUTOLYSIS ON THE SAFETY OF TISSUE-SPECIFIC PROTEINS

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Abstract – The aim of the study was to determine target tissue-specific proteins in *Bos taurus* and *Sus scrofa* heart and aorta tissues and investigate the influence of autolysis on its safety. Proteins profiles of native, frozen (minus 10°C) and autolized (4 °C for 4 days) tissues were carried out according to one-dimensional electrophoresis (Laemmli method) and two-dimensional electrophoresis (O'Farrell method). The most intensive bands were observed in the range from 100 to 10 kDa, while increased diversity and intensity of protein fractions in autolyzed samples were detected, especially in cardiac muscle. Tissue-specific proteins were detected in *Sus scrofa* aorta tissues, such as apolipoprotein A-1 (13,14), involved in the formation of HDL, peroxiredoxin 1 (10, in mixture with transgelin) involved in the suppression of oxidative stress, galectin-1 (17) induced apoptosis of T lymphocytes, and a number of heat shock proteins having a molecular weight less than 30 kDa, while in *Bos taurus* aorta tissues detected target proteins were not found or in less quantity. It was revealed that freezing preserved tissue-specific target proteins with molecular mass less than 30 kDa in *Sus scrofa* aorta tissues while during autolysis the same proteins were proteolized.

Key words: tissue-specific proteins, autolysis, electrophoresis, functional meat products

I. INTRODUCTION

Numerous strategies for improving functionality of meat and meat products are developed such as addition of functional bioactive substances and *in vivo* modification of raw meat. Recipe modifications (substitutes of fatty acids or polyunsaturated fatty acids addition, sodium chloride reduction), functional bioactive substances and modules addition (herbal ingredients (oils, extracts, fiber), soy protein, natural and synthetic antioxidants, lactic acid bacteria, fish oil, bioactive proteins and peptides isolated from farm animals organs and tissues) are successfully applied in the functional and specialized meat products processing. On the basis proteomics it makes possible to consider animal protein not only as a source of plastic material, but also as the encoded amino acid sequence. This sequence proteilized into specific peptides by enzymes the gastrointestinal tract or during fermentation and may be involved in regulatory and signaling processes, homeostasis maintenance, as well as directly or indirectly regulate the functional activity of genes, activate transcription factors and incorporate in the metabolic pathway [1-3].

For accumulation functional peptides in meat are applied different methods, including fermentation (with use of starter culture or enzyme modules), autolysis or controlling direct hydrolysis [4]. Autolytic processes in the tissues of animals after slaughter included cathepsins release from lysosomes and following activation due to acid reaction in muscle cells. Nowadays, a various endopeptidases was identified the muscle tissue, for example cathepsins B 1, D, H, L, G, and exopeptidases, such as cathepsins A, B2 and C. Lysosomal exopeptidases were found after endopeptidases and possessed more proteolysis activity. The controlled autolytic processes can be used for improving functionality and quality characteristics of raw meat, such as nutritional value of peptides content [5], while target tissue-specific target proteins could be preserved from uncontrolled degradation.

Previously *Bos taurus* and *Sus scrofa* hearts and aorta tissues were investigated by one-dimensional electrophoresis (Laemmli method) and two-dimensional electrophoresis (O'Farrell method) with following proteins identification. Tissue-specific bioactive proteins with a molecular weight in the range from 100 to 10 kDa were detected mainly in *Sus scrofa* aorta tissues. Presumably, detected proteins are involved in lipid metabolism, recovery of damaged endothelial layer, coagulation and fibrinolysis [6,7].

The aim of the study was to determine target tissue-specific proteins in *Bos taurus* and *Sus scrofa* heart and aorta tissues and investigate the influence of autolysis on its safety.

II. MATERIALS AND METHODS

The objects of study were native, frozen at minus 10°C and autolized at 4°C for 4 days *Bos taurus* and *Sus scrofa* heart and aorta tissues.

One-dimensional electrophoresis was carried out according to Laemmli method [8] in the 7.5-25% gradient SDS-PAGE with 170-10kDa standards Fermentas, (Fermentas, USA). Two-dimensional electrophoresis was carried out according to the method of O'Farrell included the application of isoelectric focusing (IEF) with following fractionation in the second direction in SDS slab gel electrophoresis with the acrylamide concentration gradient [9,10]. Protein visualization by silver nitrate staining was done as described in Kovalyov et al., 1995, 2006 [9,10].

Mass spectra were obtained on a Reflex III MALDI-TOF mass spectrometer (Bruker, Billerica, MA, USA) with a UV laser (336 nm) in the positive ion mode within the range of 500–8000 Da. During MS/MS analysis, the mass spectra of fragments were recorded on a Bruker Ultraflex MALDI TOF mass spectrometer in tandem mode (TOF-TOF), with detection of positive ions. The proteins were identified by using the Mascot software in Peptide Fingerprint mode (Matrix Science, Boston, MA, USA).

III. RESULTS AND DISCUSSION

Comparative proteome analysis of fresh, frozen and autolized samples of heart tissue revealed the primarily protein profile safety.

The most intensive bands were observed in the range from 100 to 10 kDa, while increased diversity and intensity of protein fractions in autolyzed samples were detected, especially in cardiac muscle (Fig.1).

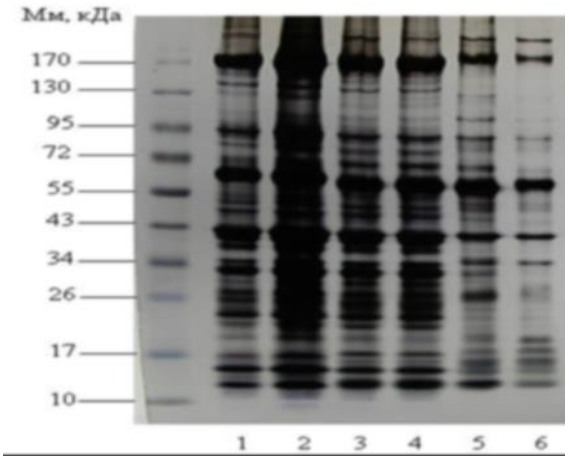
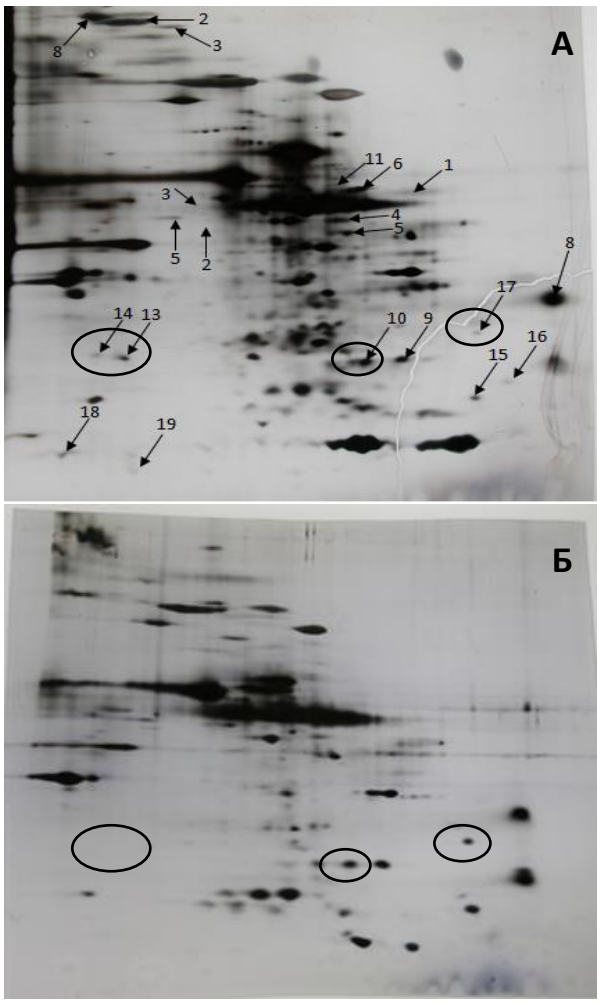


Fig. 1 Results of protein electrophoretic analysis by Laemmli method. C – standard of molecular masses (Fermentas, USA), 1 – frozen *Bos taurus* heart tissue, 2 – autolized *Bos taurus* heart tissue, 3 - frozen *Sus scrofa* heart tissue, 4 - autolized *Sus scrofa* heart tissue; 5 – autolized *Sus scrofa* aorta tissue; 6 - autolized *Bos taurus* aorta tissue

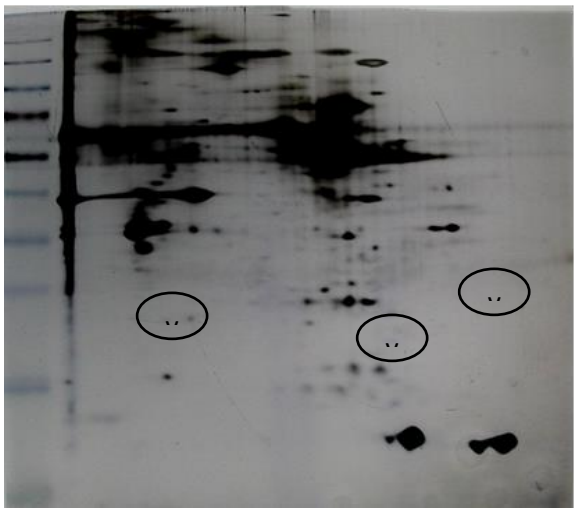
Revealed data according to 2D-electrophoresis confirmed the presence of several fractions in the most intense band in 1D-electrophoresis. Major proteins of heart muscle *Bos taurus* and *Sus scrofa* were almost identical. However, significant interspecific differences were found when comparing the proteomic profiles of the aorta samples. Moreover, target tissue-specific proteins were detected in *Sus scrofa* aorta tissues, such as apolipoprotein A-1 (13,14), involved in the formation of HDL, peroxiredoxin 1 (10, in mixture with transgelin) involved in the suppression of oxidative stress, galectin-1 (17) induced apoptosis of T lymphocytes, and a number of heat shock proteins having a molecular weight less than 30 kDa, while in *Bos taurus* aorta tissues detected target proteins were not found or in less quantity (Fig. 2).

Fig. 2 1 Results of protein electrophoretic analysis by O'Farrel method. А - *Sus scrofa* aorta tissues, Б - *Bos taurus* aorta tissues. Numeration: keratin, type II cytoskeletal 1 (1, 3); actin-related protein 3 (2); integrin-linked protein kinase isoform X1 (4); beta-enolase isoform X2 (5); prolargin isoform X1 (6); poly [ADP-ribose] polymerase 6 isoform X1 (7); four and a half LIM domains 1 protein, isoform C isoform X5 (8); transgelin (9); peroxiredoxin-1 isoformX5 (10); albumin (11); actin-related protein 3 (12); apolipoprotein A-I preproprotein (13); apolipoprotein A-I (14); calponin-1 (фрагмент 9-132); peptidyl-prolyl cis-trans isomerase B (15); calponin-1 (16); galectin-1 (17); protein S100-A13 (18).



The influence of autolysis on the safety of target tissue-specific proteins in Sus scrofa aorta tissues was also investigated(Fig. 3).

Fig. 2 Results of protein electrophoretic analysis by O'Farrel method: *Sus scrofa* autolized aorta tissues



It was shown, that after 4 days autolysis apolipoprotein A-1, peroxiredoxin 1, galectin-1 (17) as well as heat shock proteins completely or significantly proteolized.

IV. CONCLUSIONS

Various tissue-specific proteins were detected in *Sus scrofa* aorta tissues, such as apolipoprotein A-1 (13,14), involved in the formation of HDL, peroxiredoxin 1 (10, in mixture with transgelin) involved in the suppression of oxidative stress, galectin-1 (17) induced apoptosis of T lymphocytes, and a number of heat shock proteins having a molecular weight less than 30 kDa. It was also revealed that autolytic enzymes proteolyzed target tissue-specific bioactive proteins. Therefore, aorta tissues as functional additive to meat products should be applied native or frozen to preserve active components.

V. ACKNOWLEDGEMENTS

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REFERENCES

1. Hui, Y. H. (2012) Handbook of Meat and Meat Processing, Second Edition. USA: Taylor & Francis Group.

2. Lafarga, T. & Hayes, M. (2014) Bioactive peptides from meat muscle and by–products: generation, functionality and application as functional ingredients. Meat science 98: 227–239.

3. Olmedilla–Alonso, B., Jiménez–Colmenero, F. & Sánchez–Muniz, F.J. (2013) Development and assessment of healthy properties of meat and meat products designed as functional foods. Meat science 95: 919–930.

4. Sharma, S., Singh, R. & Rana, S. (2011) Bioactive Peptides: A Review. International Journal of Bioautomation 15(4): 223–250.

5. Lian, T., Wang, L. & Liu,Y. (2013) A New Insight into the Role of Calpains in Post-mortem Meat Tenderization in Domestic Animals: A review. Asian-Australas Journal of Animal Science 26(3): P. 443–454.

6. Lisitsyn, A.B., Chernukha, I.M., Fedulova, L.V., Kotenkova, E.A. & Shyshkin, S.S. (2013) Cattle and pig heart and aorta tissues as a functional food ingredient with a protein-peptide profile. Vse o myace 5: 48-51.

7. Chernukha, I.M., Fedulova, L.V. & Kotenkova, E.A. (2015) Meat By-product is a Source of Tissue-specific Bioactive

Proteins and Peptides Against Cardio-vascular Diseases. Procedia Food Science 5: 50-53.
8. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259): 680-685.
9. Kovalyov, L.I., Shishkin, S.S., Efimochkin, A.S., Kovalyova, M.A., Ershova, E.S., Egorov, T.A., & Musalyamov, A.K. (1995). The major protein expression profile and two dimensional protein database of human heart. Electrophoresis 16(7): 1160-1169.
10. Kovalyov, L.I., Kovalyova, M.A., Kovalyov, P.L., Serebryakova, M.V., Moshkovskii, S.A. & Shishkin, S.S. (2006). Polymorphism of delta3,5-delta2,4-dienoyl-coenzyme A isomerase (the ECH1 gene product protein) in human striated muscle tissue. Biochemistry (Moscow) 71(4): 448-453.