PROTEOMIC TECHNOLOGIES FOR PROTEIN ANALYSIS IN COOKED SAUSAGE

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Abstract – Proteomic technologies (two-dimensional electrophoresis, MALDI-TOF identification etc.) were applied for the study of cooked sausages produced from beef and pork at three Russian companies. Myoglobins and some other protein fractions (beta-enolases, triosophosphate isomerases, myosin light chains) were identified as species-specific proteins which may be used as potential biomarkers for protein content analysis of sausage.

Key words – biomarkers, muscular proteins, two-dimensional electrophoresis

I. INTRODUCTION

Problems of the analysis of meat products (for example, cooked sausages) for quality characteristics and revealing of falsifications require the use of not only traditional analytical methods, but also postgenomic technologies. In particular, the certain contribution to the decision of similar problems made proteomic technologies which include high-effective methods for protein fractionation, mass - spectrometric identification with use of various bioinformatic resources, etc. The productivity of proteomic technologies for the analysis of various meat products has been shown in a few publications [1, 2]. Different traditional approaches and the methods (including monitoring of actual structure, definition of presence of not muscular protein additives having a vegetative or animal origin), and also the examination of a degree of conformity to technical specifications are used in Russia to control of quality of meat products. [3]. The aim of this study is to analyse samples of cooked sausage «Doktorskaya», produced from beef and pork at three Russian companies, and of the initial meat raw materials with the application of proteomic technologies and investigate several proteins (myoglobins, beta-enolases, triosophosphate isomerases, myosin light chains) as potential biomarkers of a muscular species-specific tissues.

II. MATERIALS AND METHODS

The samples of cooked sausages «Doktorskaya» produced from beef and pork according to the same specification at three Russian companies (D1, D2, D3) were studied. The preparation of protein extracts, two-dimensional electrophoresis, protein visualization by Coomassie Blue R-250 and sodium nitrate were performed as described earlier [4].

Densitometry of two-dimensional electrophoregrams (2DE) and/or their individual fragments (rectangles) was performed after scanning (Epson Expression 1680 scanner) or filming by means of a digital photocamera (Nikon 2500 or Canon PowerShot A1000 IS). The computer handling of the resultant densitometry images of protein fractions was performed using the Melanie ImageMaster software, versions 6 and 7(Genebio, Switzerland). The statistical analysis was performed using the nonparametric Wilcoxon–Mann–Whitney test.

Some fractions chosen for identification were cut out from 2DE slab gels and the proteins were hydrolyzed by trypsin. The extracted tryptic peptides were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) as described

Govorun et al. [5]. A sample (0.5 μL) was mixed with the same volume of 20% acetonitril solution containing 0.1% of trifluoroacetic acid and 20 mg/ml 2,5-dihydroxybenzoic acid (Sigma, USA), and was air-dried. Mass-spectra were obtained on MALDI-TOF-mass-spectrometer Reflex III (Bruker, USA) with UVlaser (336 nm) in the regime of positive ions in the range 500-8,000 Da and their calibration was done in accordance with the known peaks of trypsin autolysis. During MS/MS analysis mass-spectra of fragments were registered on MALDI-TOF mass-spectrometer Bruker Ultraflex in the tandem regime (TOF-TOF) with detection of positive ions. Fragmentation of ions was induced by helium supply in the area of the initial part of the free ion drift trajectory. Accuracy of mass fragments measurements did not exceed 0.05 %. Only C-signals of terminal peptide fragments disrupted by the peptide bond (y-ions) were observed on the mass-spectrum. Protein identification was done with the help of Mascot software, option Peptide Fingerprint (Matrix Science, USA), with the accuracy of mass measurement MH+ equal to 0.01% (with a possibility to modify cysteines by acrylamide and methionine oxidation).

III. RESULTS AND DISCUSSION

Typical 2DE of proteins from cooked sausage «Doktorskaya» revealed on Fig.1. 2DE protein patterns of different analyzed samples (cooked sausage «Doktorskaya» as well as protein extracts from beef and pork) have shown the certain similarity with each other, and also appeared to some extent comparable with results of proteomic analysis of proteins from human skeletal muscles which have been published earlier [4]. In particular, on all 2DE there were some major protein fractions with electrophoretic properties, which were characteristic for such well-known proteins as actin (Act), tropomyosin (Tpm) and myosin light chains (MLC1, MLC2) identified earlier. In this study the results of protein identification on 2DE cooked sausage «Doktorskaya» summarized in Table 1.

As can see from the obtained data, among the identified protein fractions six fractions have been characterized as proteins of the pig [*Sus scrofa*], and others six - as proteins of the cow [*Bos Taurus*] (in view of them electrophoretic mobilities). Such results can be quite expected because in Russia accordingly with the normative documentation in content of cooked sausage of a grade «Doktorskaya» enter both a pork, and a beef. The species differences of corresponding protein fractions also has been confirmed at in parallel carried out the proteomic analysis of samples of a pork and a beef (including coelectrophoresis).

Detection of some quantity of milk proteins (Table 1, fractions 13 and 14) also does not contradict the accepted normative documentation. At the same time these data were shown, that proteomic technologies allow to detect non-muscular proteins in the sausage content. Accordingly, the application of proteomic technologies opens a way to detection of falsification of meat products, for example by some additives in which were contained non-muscular proteins.

Comparative proteomic analysis of three pairs of species-specific proteins (myoglobins, beta-enolases, triosophosphate isomerases, myosin light chains; Fig.1 and Table 1) in three samples cooked sausage "Doktorskaya" revealed some quantitative variability of these protein fractions. As example, Fig. 2 is shown 2DE rectangles of different samples of cooked sausages «Doktorskaya» (D1, D2, D3), on which myoglobin fractions were located, as 3D models, allowing to estimate a quantitative ratio of species-specific myoglobin isoforms. Accordingly, we expect that by results of the similar analysis it is possible to characterize a ratio of pork and beef in the sausages.

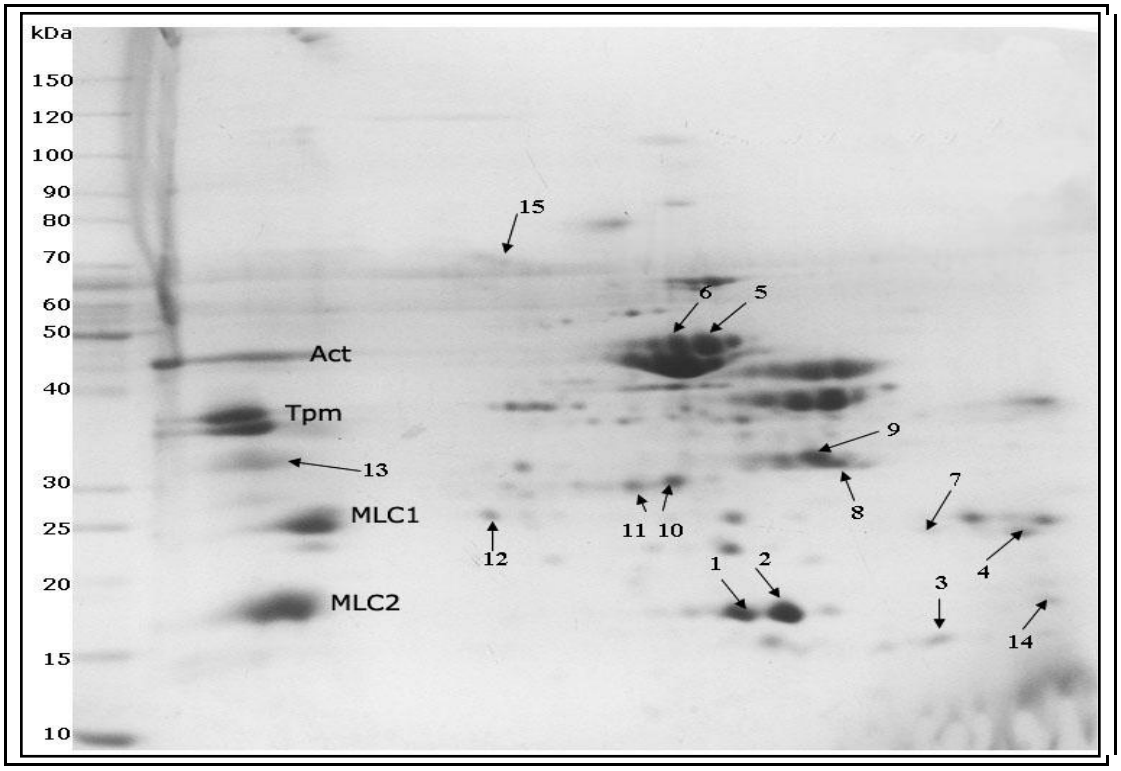

Figure 1. Typical 2DE protein pattern of cooked sausage «Doktorskaya». Coomassie Blue R-250 stain. Arrows and numbers revealed the proteins that were identified by MALDI-TOF (Results of protein identification shown in Table 1). Act – actin, Tpm – tropomyosin, MLC1 and MLC2 – myosin light chains, respectively (explanation in the text).

Table 1. Results of protein identification on 2DE of extracts from sausage «Doktorskaya»

№	Name of protein	NCBI accession no.	Score / No. of peptide matched	Coverage	Experi-mental M_r/pI.	Theoretical M_r/pI, using ExpASY tool
1	Myoglobin (*Sus scrofa*)	47523546	157/13	77	17,0/7,75	17,1/6,76
2	Myoglobin (*Bos taurus*)	27806939	146/13	75	17,0/6,90	17,7/6,90
3	Hemoglobin alpha (*Sus scrofa*)	229626	91/9	58	15,0/8,70	15,0/8,73
4	Troponin I type 2 skeletal, fast (*Bos taurus*)	300797481	59/10	39	22,0/8,90	21,4/8,88
5	Beta-Enolase (*Sus scrofa*)	113205498	343/30	66	46,5/6,80	47,1/8,05
6	Beta-Enolase (*Bos taurus*)	77736349	260/31	61	46,5/6,70	47,1/7,60
7	Troponin I type 2 skeletal, fast (*Bos taurus*)	300797481	172/21	57	22,0/8,80	21,4/8,88
8	Phosphoglycerate mutase 2 (*Sus scrofa*)	201066358	284/28	74	30,0/7,60	28,7/8,86
9	Carbonic anhydrase 3 (*Sus scrofa*)	56711366	153/11	47	30,5/7,55	29,4/7,72
10	Triosephosphate isomerase 1 (*Sus scrofa*)	262263205	388/24	94	27,0/6,50	26,7/6,45
11	Triosephosphate isomerase 1 (*Bos taurus*)	61888856	246/18	79	26,5/6,40	26,7/6,45
12	Myosin light chain 6B (*Bos taurus*)	115496556	148/13	61	24,0/5,40	23,4/5,40
13	Beta-casein, CSN2 (*Bos taurus*)	83406093	97/9	29	33,0/4,80	25.2/5.53
14	Casein para kappaA (*Bos taurus*)	229416	94/6	77	14,0/8,75	12,3/8,92
15	Serum albumin precursor (*Bos taurus*)	30794280	140/21	34	67,0/5,60	67,0/5,71

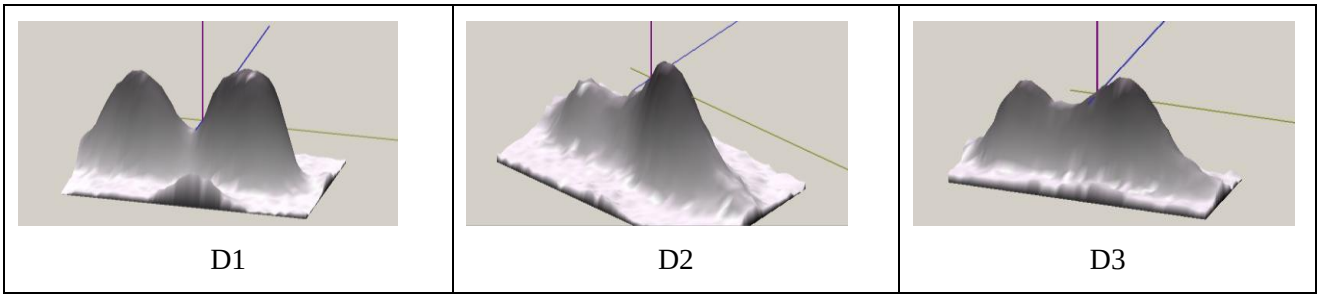

Figure 2. 2DE rectangles of different samples of cooked sausages «Doktorskaya» (D1, D2, D3), on which myoglobin fractions were located, as 3D models, allowing to estimate a quantitative ratio of species-specific myoglobin isoforms.

IV. CONCLUSIONS

Summarizing the obtained data, it may be concluded that proteomic technologies can be applied to the analysis of muscular proteins of cooked sausage and evidently of other meat products. Moreover, the application of proteomic technologies opens a way to detect fake meat products, if some unpermitted additives containing non-muscular proteins were used.

It was revealed that some identified muscular proteins (myoglobins, beta-enolases, triosophosphate isomerases, myosin light chains) can be used as potential biomarkers of a muscular species-specific tissues into meat products.

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