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COMPARISON OF THE PROTEOMIC PROFILE OF PORK BY-PRODUCTS DURING THEIR STORAGE

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Abstract
In this article, the proteomic profiles of pork by-products (snout, tongue, liver, kidney, spleen) were studied by comparative method on the first day and the fifth day of their storage. Two-dimensional electrophoresis according to O'Farrell was used for the aims of this article, while the results were further processed in ImageMaster software. Proteomic maps of by-products showed clear changes in protein composition after visualization and images analysis. There was a decrease and increase in manifestation intensity of some proteins. The study of the obtained electrophoregrams with the help of references resources allowed identifying various compounds in the by-products. 9 protein fractions with various intensity of manifestation were found on the day 1st and 5th. On the 1st day the following substances were intensively manifested: in the liver — glutathione peroxidase 4 (22.3 kDa), LEAP-2 (8.8 kDa); in the kidneys — quinone oxidoreductase (34.9 kDa); in the spleen — glycoprotein CD59 (13.7 kDa), in the patch — protein flint (49.07 kDa). It is noted that these proteins play their role in stopping certain processes in cells, like oxidation, microbial activity, and accumulation of toxic substances. These processes can worsen the quality of raw materials, and further lead to spoilage of the food product. On the 5th day of storage the highest intensity of manifestation of glyceraldehyde-3-phosphate dehydrogenase (35.8 kDa) in the liver was observed; superoxide dismutase [Cu-Zn] (15.8 kDa) was noted in the kidneys, colony-stimulating factor (16.2 kDa) was observed in the spleen and glutaredoxin –1 (11.8 kDa) in the tongue. In its turn, on the fifth day these chemical processes manifested themselves more intensely, as the fatty acids and glucose broke down. To obtain more accurate results, the proteins were compared by their volume. Among the identified fractions the highest expression was observed in LEAP 2 (8.8 kDa) on the first day, and in glyceraldehyde-3-phosphate dehydrogenase (35.8 kDa) on the fifth day. The least change in the intensity of manifestation was noted for superoxide dismutase [Cu-Zn] (15.8 kDa), which volume increased during storage by 13% for 5 days. The analysis of the obtained electrophoregrams allowed identifying various compounds, tracing the changes in the qualitative composition of protein in by-products during various periods of their storage. The obtained data demonstrate the transformation of protein molecules during storage, which makes it possible to determine the changes and quality of the food products.

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Introduction

In the modern world the composition and biological value of meat food is of great importance, as the meat food products are the main source of animal protein and provide a significant impact on human health. Meat products are very closely related to the human diet and its taste needs, which can change over time in direction of some certain types of meat. That is why the variety of choice of types of meat products, as well as the expansion of people's taste preferences, have led to great attention of producers and consumers to by-products as type of raw material.

If we consider the additional source of nutrients, and most importantly protein, then recently there has been an increase in the demand for by-products, which, in their measure, are able to provide people with a daily need for protein, vitamins and minerals, as well as to diversify their diet with various dishes [1]. In terms of nutritional value the pork by-products are not inferior to meat due to their greater variety, and the amount of various trace elements and vitamins, compared to muscle tissue, is many times greater. For example the liver contains a lot of zinc, copper, magnesium and potassium, and pork kidneys contain a large amount of sodium and calcium [2, 3]. The pork tongue is rich in protein, fat, and significant amounts of iron and zinc [4]. As for vitamins, based on studies, it has been noted that their amount is greater than in muscle tissues [5]. For example, the liver contains a large amount of vitamins A and D, as well as many B

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vitamins, the tongue is rich in choline and vitamin B12, and pork spleen is rich in A and B3. According to the data on various trace elements and vitamins contained in the by-products, it can be concluded that by-product can serve as an alternative to raw meat, being the source of many trace elements [6].

By-products are sold to consumers, both in original and processed form. The following types of meat products are very popular: various types of sausages (liver sausages, blood sausages, pates), canned food. The most valuable and delicious types of by-products include the tongue, liver, heart and kidneys; in terms of nutritional value they are equivalent to meat [7].

In regards to the nutritional values, to taste and other benefits of by-products, it is necessary to keep in mind such an important factor as the very quality of the product. The quality of raw materials is one of the main priorities in the food industry. In the aggregate it consists of appearance, taste, smell, but the most important thing is the composition of the by-product: the amount of proteins, fats, carbohydrates, minerals and amino acids. All these parameters depend on many factors: method of an animal feeding, feeding conditions, pre-slaughter processing, primary processing and storage conditions.

By-products are quite often used by consumers for cooking of whole variety of dishes. The use of by-products is often caused by their lower cost in comparison with raw meat, and therefore their greater availability. At the same time, there is little information about the changes which occur in the protein profiles of pork by-products over time. During the storage period, they are subject to changes in organoleptic properties, nutritional value and protein composition [8, 9].

To date of great interest are the works that help to determine and analyze the rich proteomic composition of consumed food products of animal and plant origin. Proteomics opens up great opportunities for researchers for the study of this topic. Using of proteomics allows identifying proteins in products, characterizing them, and comparing the proteomic composition of meat raw materials obtained from various animal species [10]. The main proteomics tool that remains relevant to this day is two-dimensional electrophoresis, which is used to study changes which occur to proteins and to identify functional species-specific and tissue-specific compounds [11]. The use of two-dimensional electrophoresis technology makes it possible to define thousands of proteins with high resolution, and characterize the defined protein fractions by mass spectrometric methods. The obvious advantage of this proteomic approach over other methods is explained by its ability to detect alternative protein forms that result from co- and/or post-translational modifications.

Another advantage of proteomic methods is its ability to track changes in the protein composition after slaughter, to assess the influence of storage on quality and food safety of by-products, because they have pretty short shelf life due to increased content of moisture and microorganisms [12,13]. Due to proteomic technologies there is a growing understanding of the various biological processes that define meat quality. This study determines the protein composition and runs comparative analysis of two-dimensional electrophoregrams of various by-products in order to trace qualitative changes during their storage.

Objects and methods

The pork by-products served as the objects of this study, in particular: chilled tongue, liver, spleen, kidneys and snout, were taken at the stage of primary processing of slaughtered pigs with a live weight of 120 kg (3 samples of each type were studied). By-product samples for research were taken from 3 animals on the first day and the fifth day of storage at a temperature of 0 to 2 °C.

Immediately before the study, a sample of 100 mg was taken and 2000 μl of a lyse solution (9 M urea, 5% β-mercaptoethanol, 2% Triton X-100, 2% ampholine pH 3–10) was added. The resulting homogenate was clarified by centrifugation at 14,000 rpm for 20 minutes. Further, protein extracts were used in isoelectric focusing.

During study of the samples, the method of two-dimensional electrophoresis (2-DE) according to O`Farrell using isoelectric focusing (IEF) was used [14, 15]. IEF was carried out in tubular gels at 3,650 V/h. After isoelectric focusing, the resulting gels were kept in balancing buffers for 10 min each [16].

Next, the gels were exposed to electrophoresis with sodium dodecyl sulfate; for this, the balanced gels were transferred into a 12.5% polyacrylamide gel. Electrophoresis was run using a buffer containing 25 mM Tris-HCl, 192 mM glycine and 0.1% SDS in amount of 30 mA per gel until the stain front reached the edge of the gel.

The protein zones after their electrophoretic separation in polyacrylamide gel (PAAG) were stained and localized using Coomassie G-250. Each sample was presented in triplicate.

Computer densitometry of two-dimensional wet electrophoregrams was performed using a scanner *Bio-5000 plus* (Serva, Germany), resolution 300 ppi 1D-Gray. The resulting images were analyzed using ImageMaster™ 2D Platinum software based on Melanie 8.0 (GE Healthcare and Genebio, Switzerland). Next, the digitized images were compared by the method of matching. The visualized protein stains were interpreted using the UniProt database [17].

Further, the obtained experimental data were analyzed using Student's t-test and one-way analysis of variance (between gels of different samples) using ImageMaster ™ 2D Platinum software based on Melanie 8.0 (GE Healthcare and Genebio, Switzerland). It was considered that P value < 0.05 indicates a significant difference. As part of the work, protein stains were compared by their volume and the Fold index was calculated, the excess of which by more than 2 units is generally considered to be a statistically significant difference. All results are presented as mean ± standard deviation from at least three independent trials.

Results and discussion

The study was run on samples of the pork by-products: snout, tongue, liver, spleen and kidney at various periods of storage (the first day and the fifth day) in order to identify significant changes in the protein composition during their storage. A wide range of various protein compounds with molecular masses from 7 kDa and higher was obtained by the method of two-dimensional electrophoresis. As a result of analysis of 2-DE gel images by ImageMaster™ 2D Platinum, about 110 different protein fractions in average were found in each by-product. At the same time, the highest content of proteins was in the liver, kidneys, spleen, and the lowest — in the patch.

Figures 1 and 2 below represent the obtained electrophoregrams of the by-products. When analyzing the proteomes, the researcher can notice differences such as a decrease in intensity of protein manifestation by the fifth day and occurrence of new protein structures that were not found on the first day. Comparative analysis of electrophoregrams showed that the smallest amount of protein stains was revealed in the samples of a snout compared to other by-products, while an increase in the amount of proteins was observed by the fifth day.

When comparing the electrophoregrams of by-products analyzed on the first day and the fifth day of their storage, a difference was revealed in their proteomic profile and in manifestation of individual proteins. Protein fractions were identified, in which the intensity decreased on the fifth day (No. 1 — No. 5 in Figure 3), among which can be noted in liver samples — glutathione peroxidase 4 (22.3 kDa), which protects the cell from oxidative damage and LEAP-2 (8.8 kDa), which has antimicrobial activity [18,19]; kidneys — quinone oxidoreductase (34.9 kDa), which is involved in detoxification of xenobiotics and reduces the load of free radicals in cells [20]; spleen — glycoprotein CD59 (13.7 kDa), which is a powerful inhibitor of action of the complementary membrane attack complex [21]; snout is a kremen protein (49.07 kDa), which can cause cell death, being an addiction receptor [22]. It was noted that the functions of the above described proteins are mainly aimed at protecting cells from various types of damage. It is highly likely that processes started in the by-product cells which adversely affect the composition and quality of meat raw materials, as well as lead to its deterioration.

Among the identified fractions, spots of proteins were noted with high expression by the fifth day (No.6 — No.9 in Figure 4). For liver samples, glyceraldehyde-3-phosphate dehydrogenase (35.8 kDa) was found to be involved in glycolysis [23]; in the kidneys — superoxide dismutase [Cu-Zn] (15.8 kDa), which destroys toxic radicals [24]; in the spleen, a colony-stimulating factor (16.2 kDa) necessary for β-oxidation of fatty acids [25]; in the tongue — glutaredoxin-1 (11.8 kDa), which reduces the content of glycosylated proteins, and is also an antioxidant enzyme [26]. In cells on the 5th day, more intense chemical processes are observed associated with a decrease in the quality of the product, such as glycolysis, the formation of toxic radicals, and β-oxidation of fats.

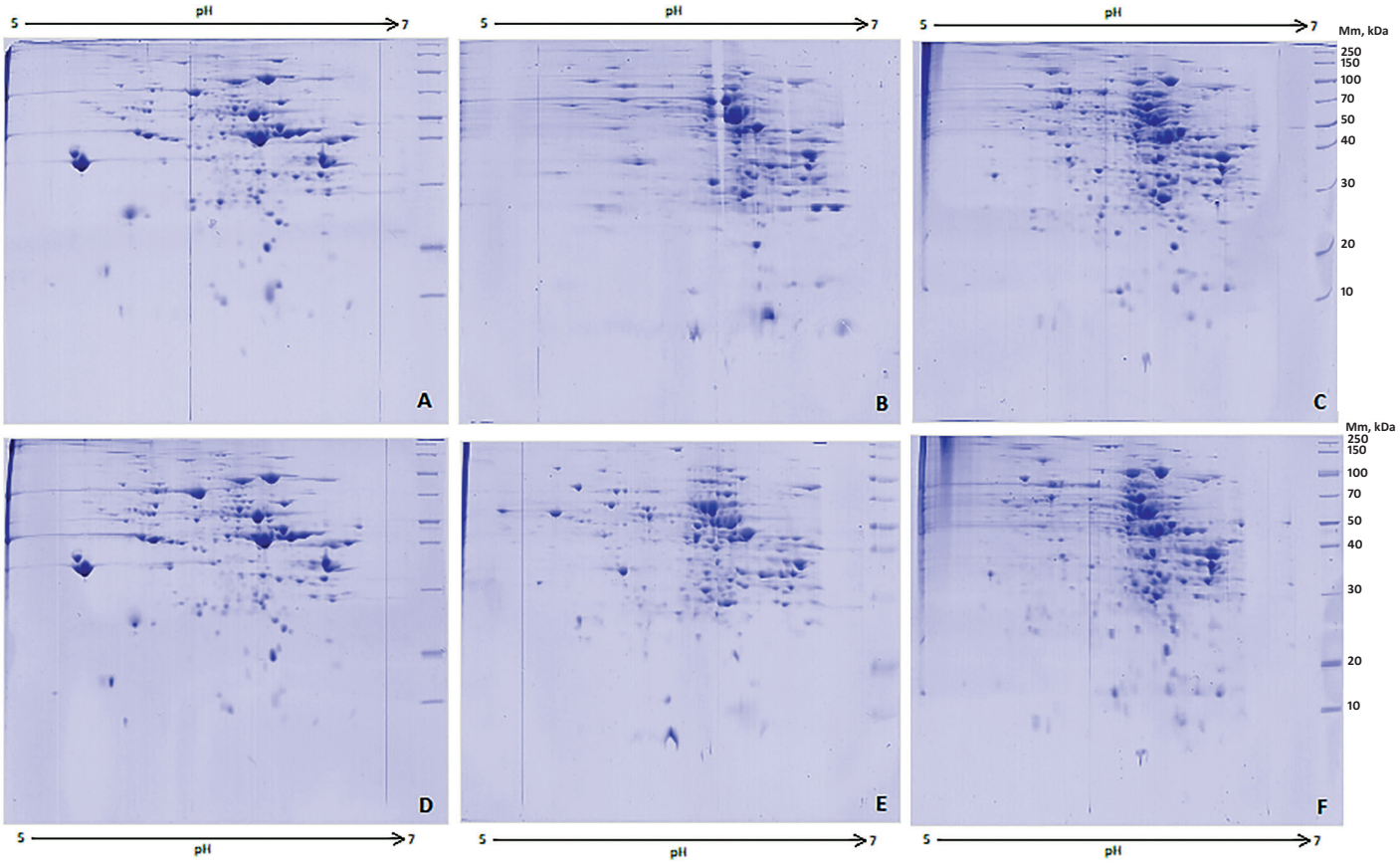

Figure 1. Two-dimensional electrophoregrams of the spleen (A — on the first day, D — on the fifth day), liver (B — on the first day, E — on the fifth day) and kidney (C — on the first day, F — on the fifth day)

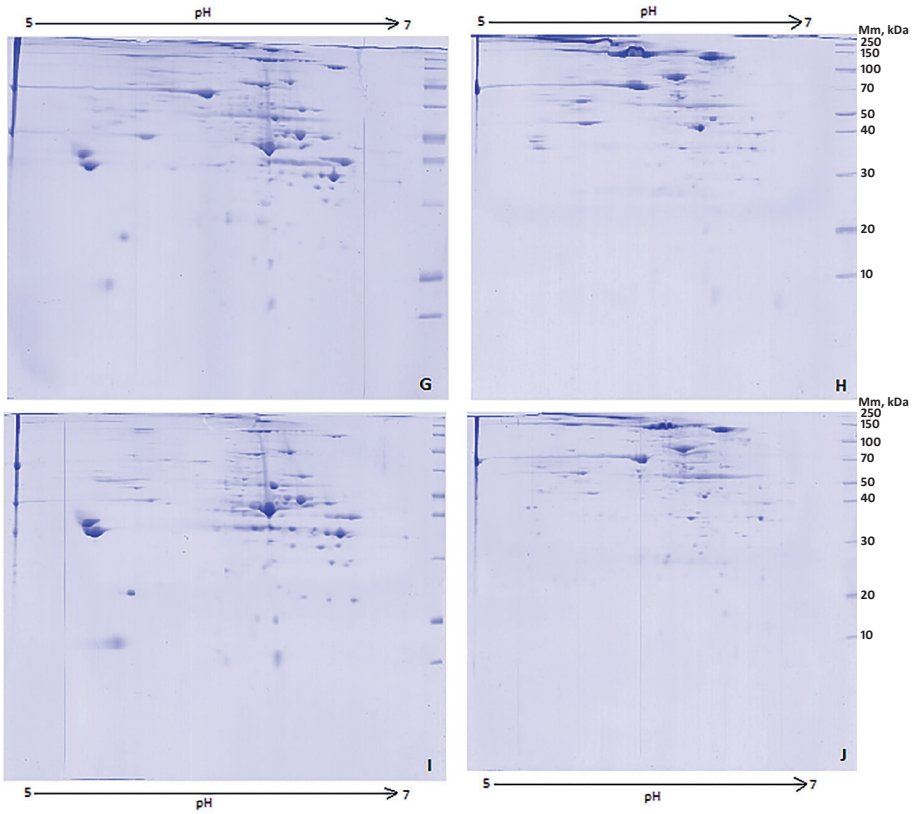

Figure 2. Two-dimensional electrophoregrams of the tongue
(G — on the first day, I — on the fifth day) and patch (H — on the first day, J — on the fifth day)

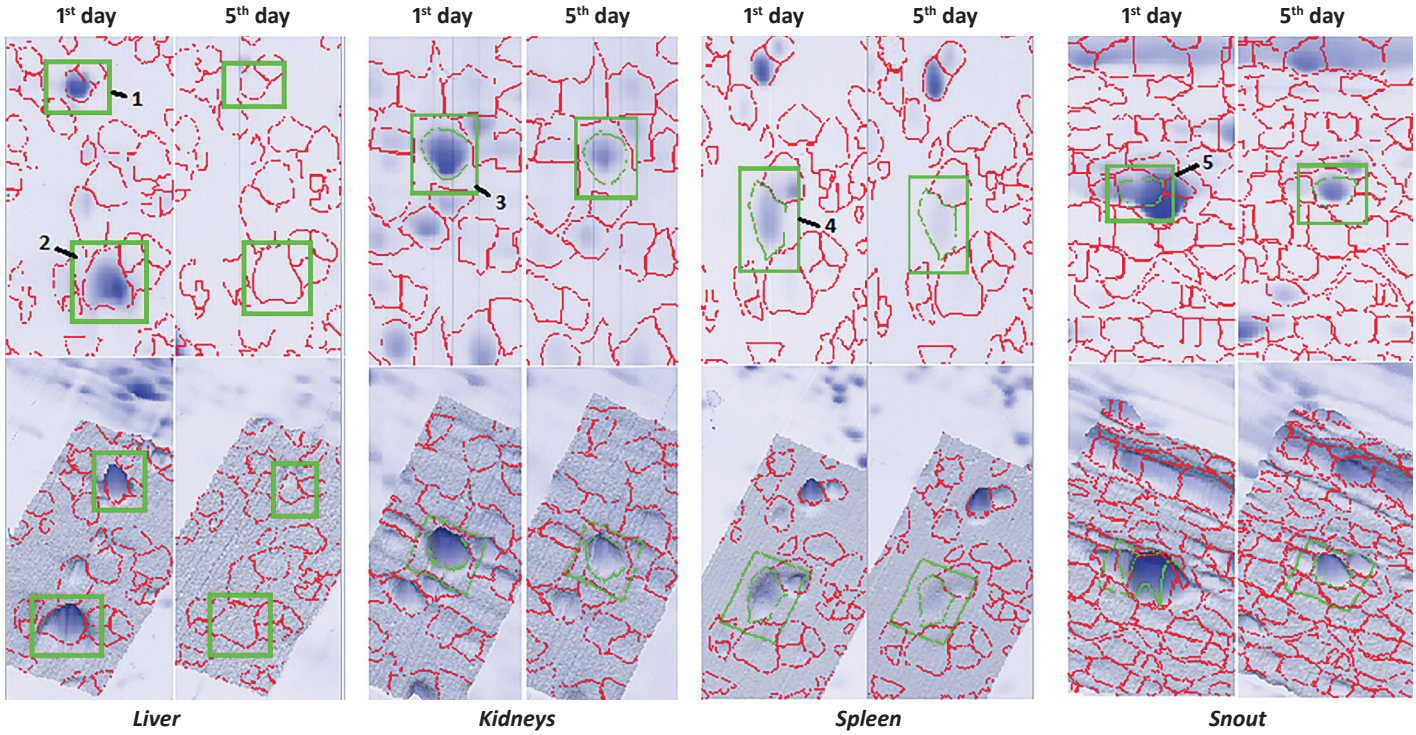

Figure 3. Fragments of 2-DE gels of pork by-products on days 1 and 5

As a further part of the research, in order to compare the intensity of protein manifestation in certain days, protein stains were compared by their volume (Figure 5).

In proteins No. 3 and No. 4, the saturation of staining evenly decreased by 43% and 50% from days 1 to 5 respectively, and in protein No. 7 it increased by 12%. Presumably, the decrease in the intensity of protein No. 3 was caused by the neutralization of free radicals and increase in toxic substances, and therefore the increase of protein No. 7 volume. The largest difference in optical density is observed in fraction No. 2 — on the first day it was 10 times denser than on the fifth day. Further, proteins No. 6 and No. 9 can also be noted, in which the volume increased by 5 times in comparison with the first day. Protein No. 6 is probably glyceraldehyde-3-phosphate dehydrogenase (35.8 kDa), required for glycolysis. It can be suggested that on the 5th day of the

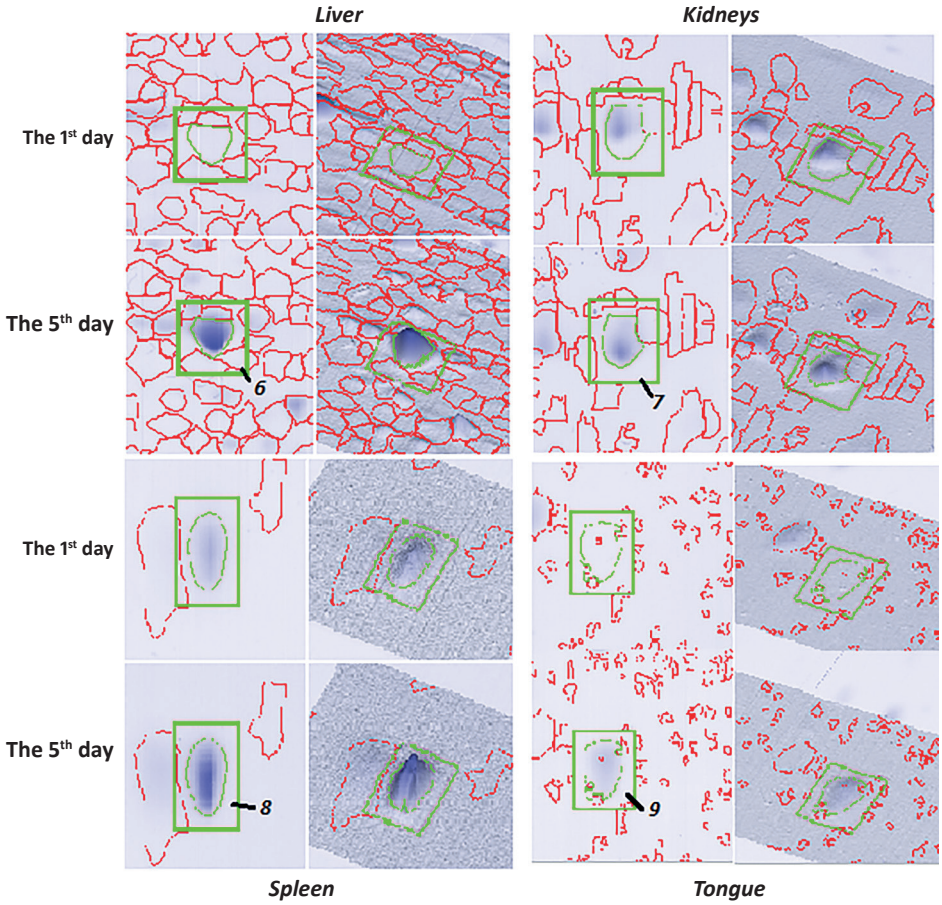

Figure 4. Fragments of 2-DE gels of pig by-products on days 1 and 5

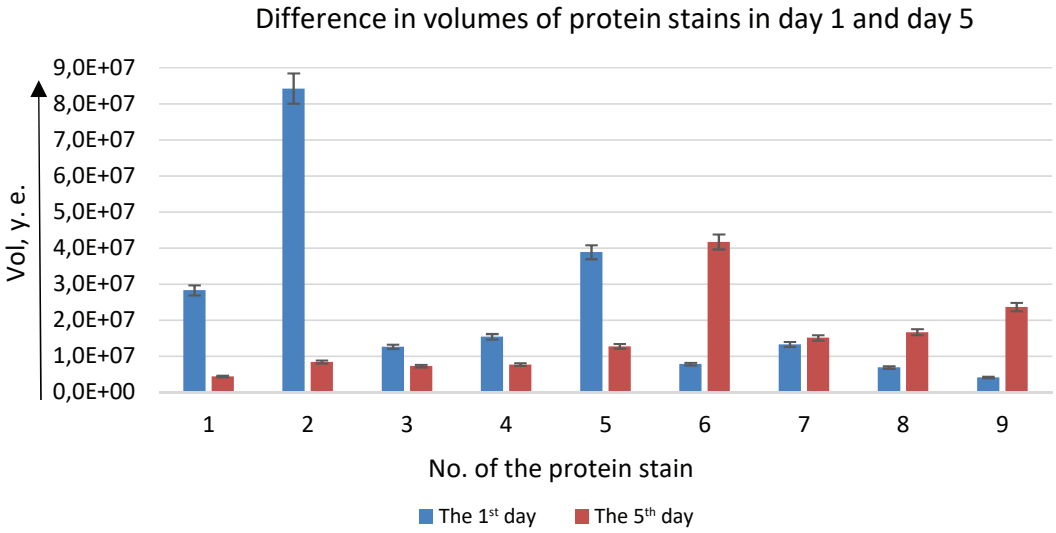

Figure 5. Integrated optical density of protein fractions

Note: Spot intensity was normalized by total spot intensity and the average of three analytical replicate gels.

by-product storage, an intensive process of glucose oxidation occurs, therefore, a large amount of lactic acid appears in the liver cells. Also, high staining intensity is observed in proteins No. 1 and No. 5 on the first day, which is respectively 6 and 3 times higher than the staining volume compared to the fifth day, and for protein No. 8 high intensity is noted by the fifth day, namely, it is higher by 59% more than in the first day.

The above listed changes in volume of protein fractions can reflect various processes which run in the cell over several days of storage. The decreased mechanisms of defense and increasing of chemical processes and reactions lead to deterioration of quality of the analyzed product. For example, protein No. 1, which is 85% more intense on the first day, is responsible for reduction of hydroperoxide groups (–OH) of fatty acids in membrane phospholipids. These changes may indicate the beginning of cell membranes destruction, which may lead to other changes in cells that affect the spoilage of the meat by-products.

Conclusion

This study allowed determining and comparing the protein composition of by-products at various periods of their storage, as well as it allowed considering the intensity of processes over time of storage. There were clear changes in the electrophoregrams from the first day to the fifth day. The most interesting were the proteins in the kidneys: quinone oxidoreductase (34.9 kDa) and superoxide dismutase [Cu-Zn] (15.8 kDa), where intensity of one protein decreased while the intensity of the other protein increased.

In the liver two protein stains were found, which intensity greatly decreased by the fifth day. Glutathione peroxidase 4 with a molecular weight of 22.3 kDa plays a key role in glycolysis. Presumably, the concentration of glucose in the cells dropped sharply over the course of 5 days in a row, thereby reducing the concentration of this protein, as was shown on fragments of 2-DE gels and on the graph of integrated optical density. The 10-fold decrease in intensity of the LEAP-2 protein (8.8 kDa) by the fifth day, which protein is responsible for antimicrobial activity, contributed to acceleration of microbiological deterioration of the liver samples.

By the fifth day decrease was noted in spleen protein glycoprotein CD59 (13.7 kDa) concentration. This protein is responsible for preservation of cell membranes. This decrease indicates cells destruction. In addition, on the fifth day the colony-stimulating factor (16.2 kDa) grew noticeably, which indicates an increase of fats β-oxidation intensity due to increase of amount of lipid peroxidation products, which include aldehydes, ketones, etc. Formation of above listed compounds leads to deterioration of organoleptic characteristics and nutritional value of by-products.

On the fifth day the amount of some proteins increased actively, namely: in the samples of snout and tongue. In the sample of tongue glutaredoxin-1 was intensely expressed, which protein is an antioxidant enzyme. This fact suggests the formation of a large number of oxidants.

The study showed that on the first day the processes start in the by-products, that negatively affect the quality of raw materials; and by the fifth day the processes begin to develop more intensively. By-products have been proved to be a rich source of protein components with a very short shelf life.

REFERENCES

1. Nasonova, V.V. (2018). Perspective ways the use of by-products. *Theory and practice of meat processing*, 3(3), 64–73. https://doi.org/10.21323/2414–438X-2018–3–3–64–73 (In Russian)
2. Kabulov B., Kassymov S., Moldabayeva Zh., Rebezov M., Zinina O., Chernyshenko Yu. et al. (2020). Developing the formulation and method of production of meat frankfurters with protein supplement from meat by-products. *EurAsian Journal of BioSciences,* 14(1), 213–218.
3. Babicz, M., Kropiwiec, K., Szyndler-Nedza, M., Skrzypczak, E. (2018). The physicochemical properties of by-product from Puławska gilts in relation to carcass meatiness. *Annals of Animal Science*, 18(1), 239–249. https://doi.org/10.1515/aoas-2017–0018
4. Biel, W., Czerniawska-Piątkowska, E., Kowalczyk, A. (2019). By-product Chemical Composition from Veal, Beef, and Lamb Maintained in Organic Production Systems. *Animals,* 9(8), Article 489. https://doi.org/10.3390/ani9080489
5. Alao, B. O., Falowo, A. B., Chulayo, A., Muchenje, V. (2017). The potential of animal by-products in food systems: Production, prospects and challenges. *Sustainability*, 9(7), Article 1089. https://doi.org/10.3390/su9071089
6. Babicz, M., Kasprzyk, A., Kropiwiec-Domańska, K. (2018). Influence of the sex and type of tissue on the basic chemical composition and the content of minerals in the sirloin and by-product of fattener pigs. *Canadian Journal of Animal Science*, 99(2), 343–348. https://doi.org/10.1139/cjas-2018–0085
7. Abdilova, G., Rebezov, M., Nesterenko, A., Safronov, S., Knysh, I., Ivanova, I. et al. (2021). Characteristics of meat by-products: nutritional and biological value. *International Journal of Modern Agriculture*, 10(2), 3895–3904.
8. Kupaeva, N.V., Kotenkova, E.A. (2019). Analysis of the antioxidant capacity of farm animal raw materials. *Vsyo o myase*, 5, 34–37. https://doi.org/10.21323/2071–2499–2019–5–34–37 (In Russian)
9. Kotenkova, E. A., Kupaeva, N. V. (2020). Assessment of the antioxidant potential of some porcine by-products from slaughter. *Food Industry*, 7, 34–40. https://doi.org/10.24411/0235–2486–2020–10073 (In Russian)
10. Akhremko, A., Vasilevskaya, E., Fedulova, L. (2020). Adaptation of two-dimensional electrophoresis for muscule tissue analysis. *Potravinarstvo Slovak Journal of Food Sciences,* 14(1), 595–601. https://doi.org/10.5219/1380
11. Banerjee, R., Maheswarappa, N.B., Mohan, K., Biswas, S., Batabyal, S. (2022). Proteomic technologies and their application for ensuring meat quality, safety and Authenticity. *Current Proteomics*, 19(2), 128–141. https://doi.org/10.2174/1570164618666210114113306
12. Chernukha, I.M., Akhremko, A.G. (2018). Application of proteomic tools: the autolytic changes of pork muscular tissue. *Theory and Practice of Meat Processing*, 3(4), 32–37. https://doi.org/10.21323/2414–438X-2018–3–4–32–37 (In Russian)
13. Zamaratskaia, G., Li, S. (2017). Proteomics in meat science — current status and future perspective. *Theory and Practice of Meat Processing*, 2(1), 18–26. https://doi.org/10.21323/2414–438X-2017–2–1–18–26 (In Russian)
14. Aryuzina, M.A., Vetrova, E.S. (2021). Application of two-dimensional electrophoresis in the study of blood plasma of biomodels. *Food Systems*, 4(3S), 8–11. https://doi.org/10.21323/2618–9771–2021–4–3S-8–11 (In Russian)
15. Ahremko, A.G. (2019). Application of two-dimensional electrophoresis method for assessing meat products composition on model stuffing example. *Vsyo o myase,* 3, 46–48. https://doi.org/10.21323/2071–2499–2019–3–46–48 (In Russian)
16. Akhremko, A. G., Vetrova, E. S. (2021). Comparative proteomic study of pig muscle proteins during growth and development of an animal. *Theory and Practice of Meat Processing,* 6(4), 320–327. https://doi.org/10.21323/2414–438X-2021–6–4–320–327
17. Data base "UniProt". Retrieved from https://www.uniprot.org/. Accessed December 23, 2021
18. Cozza, G., Rossetto, M., Bosello-Travain, V., Maiorino, M., Roveri, A., Toppo, S. et al. (2017). Glutathione peroxidase 4-catalyzed reduction of lipid hydroperoxides in membranes: the polar head of membrane phospholipids binds the enzyme and addresses the fatty acid hydroperoxide group toward the redox center. *Free Radical Biology and Medicine*, 112, 1–11. https://doi.org/10.1016/j.freeradbiomed.2017.07.010
19. Hong, Y., Truong, A. D., Lee, J., Lee, K., Kim, G. -B., Heo, K. -N. et al. (2019). Identification of duck liver-expressed antimicrobial peptide 2 and characterization of its bactericidal activity. *Asian-Australasian Journal of Animal Sciences*, 32(7), 1052–1061. https://doi.org/10.5713/ajas.18.0571
20. Pey, A. L., Megarity, C. F., Timson, D. J. (2019). NAD(P)H quinone oxidoreductase (NQO1): an enzyme which needs just enough mobility, in just the right places. *Bioscience Reports*, 39(1), Article BSR20180459. https://doi.org/10.1042/BSR20180459
21. Das, N., Anand, D., Biswas, B., Kumari, D., Gandhi, M. (2019). The membrane complement regulatory protein CD59 and its association with rheumatoid arthritis and systemic lupus erythematosus. *Current Medicine Research and Practice*, 9(5), 182–188. https://doi.org/10.1016/j.cmrp.2019.07.013

22. Sumia, I., Pierani, A., Causeret, F. (2019). Kremen1-induced cell death is regulated by homo-and heterodimerization. *Cell Death Discovery*, 5(1), Article 91. https://doi.org/10.1038/s41420–019–0175–5
23. Sirover, M. A. (2021). The role of posttranslational modification in moonlighting glyceraldehyde-3-phosphate dehydrogenase structure and function. *Amino Acids*, 53(4), 507–515. https://doi.org/10.1007/s00726–021–02959-z
24. Bakavayev, S., Chetrit, N., Zvagelsky, T., Mansour, R., Vyazmensky, M., Barak, Z. et al. (2019). Cu/Zn-superoxide dismutase and wild-type like fALS SOD1 mutants produce cytotoxic quantities of H_2O_2 via cysteine-dependent redox short-circuit. *Scientific Reports*, 9(1), Article 10826. https://doi.org/10.1038/s41598–019–47326-x
25. Wessendarp, M., Watanabe-Chailland, M., Liu, S., Stankiewicz, T., Ma, Y., Kasam, R.K. et al. (2022). Role of GM–CSF in regulating metabolism and mitochondrial functions critical to macrophage proliferation. *Mitochondrion*, 62, 85–101. https://doi.org/10.1101/2021.02.10.430444
26. Burns, M., Rizvi, S.H.M., Tsukahara, Y., Pimentel, D. R. Luptak, I., Hamburg N. M. et al. (2020). Role of glutaredoxin-1 and glutathionylation in cardiovascular diseases. *International Journal of Molecular Sciences*, 21(18), 1–17, Article 6803. https://doi.org/10.3390/ijms21186803

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