Adaptation of the two-dimensional electrophoresis method for canned meat

V B Krylova¹, V T Gustova¹ and A G Akhremko¹

¹ V.M. Gorbatov Federal Research Centre for Food Systems of Russian Academy of Science, Talalikhina str., 26, Moscow, 109316, Russia

E-mail a.akhremko@fncps.ru

Abstract. Studies of the qualitative indicators of canned meat in accordance with regulatory documents are carried out on average samples of specimens, but when studying by proteomic methods, such sampling does not allow high-quality separation of protein components due to the high fat content in the product. When two-dimensional electrophoresis was carried out on an average sample, fragments of the main muscle and connective tissue proteins of beef were found in small quantities, but the electrophoretogram was not very informative. A significantly better separation was achieved after removing the fat fraction from the product. When studying broth from canned meat, the largest amount of intensely coloured high-molecular-weight protein fractions with a mass of more than 50 kDa was revealed. The electrophoretogram of the meat pieces showed a wide range of proteins across the entire molecular weight range of the polyacrylamide gel, including major muscle proteins. The study of broth together with meat pieces but after fat removal is optimal for the primary screening of the protein component of canned meat.

1. Introduction

Animal foods are among those foods that contain many important nutrients. The food industry uses many technologies to produce products with a variety of shelf life durations. There are already adapted methods for determining the proteomic profile of meat and some meat products. However, processing conditions and methods can change the nutritional value, texture and taste of meat, which makes it difficult to identify proteins in cooked foods [2]. The high temperature processing of the product contributes to the destructive changes in the meat system, which has both positive and negative effects on the nutritional value of products [3]. Understanding the functional mechanisms of protein systems at the molecular level will make it possible to control these processes, possibly also to correlate the risky technological aspects of industrial food production. The range of proteomic technologies can be divided into several main groups, presented in Figure 1. Proteomics techniques complement existing methodologies for quality assurance and food safety authentication. The protein complex of food products is analysed using a variety of high performance separation methods such as one-dimensional and multivariate chromatography, two-dimensional gel electrophoresis, and high-resolution mass spectrometry. The use of these methods makes it possible to control the protein composition of food products and their changes during the production process, while their use is also invaluable in the authentication of toxins, allergens, nutritional value and shelf life or during storage [4].
Figure 1. The basic approaches and methods for proteomics. 1DE - one-dimensional electrophoresis; 2DE - two-dimensional electrophoresis; 2-D DIGE - two-dimensional differential electrophoresis; LC - high performance liquid chromatography; MDLC multidimensional LC; MALDI - matrix-activated laser desorption / ionisation; MS - mass spectrometry; MS / MS - tandem MS; PTM - post-translational modifications; SELDI - surface enhanced laser desorption / ionisation.

One of the most famous methods for obtaining proteomic profiles belonging to the “bottom-up” group is two-dimensional electrophoresis of proteins [5]. To separate protein extracts in the first direction, isoelectric focusing in thin columns of polyacrylamide gel (PAGE) is used, with which proteins are separated depending on the value of their isoelectric points. As a method used in the separation of proteins in the second direction, PAGE gradient plate electrophoresis was performed in the presence of sodium dodecyl sulphate as an ionic detergent. Two-dimensional electrophoresis makes it possible to analyse complex protein mixtures and produces electrophoretograms that show more than 1000 fractions [6]. Despite some limitations, mainly related to sensitivity and reproducibility, two-dimensional electrophoresis is used for the systematic study of a wide variety of biological objects [7, 8, 9, 10, 11]. Optimisation of protocols for sample preparation during protein extraction and solubilisation allows these problems to be solved. With that in mind, the purpose of this study was to adapt the method of two-dimensional electrophoresis to determine the protein composition of canned meat.

2. Materials and methods
As an object of research, we took sterilised consumer packages of the canned meat, “Top grade stewed beef”. The ingredients of this canned food were: beef, beef fat, onions, salt and bay leaf.

To produce an average sample, the lids of the cans were cut with a knife around 3/4 of the circumference, and, bending them slightly outward so that the solid parts of the canned food did not pass through the gap, the liquid part was poured into a porcelain cup. The solid part of the canned meat was passed twice through a meat grinder, mixed with the liquid part and ground in parts in a porcelain mortar until a homogeneous mass was achieved, which was then used for further analysis.

The other samples for this study were produced using a modified sample protocol, as described below.

The protein composition of the canned meat system was analysed by the method of two-dimensional electrophoresis according to O’Farrell with isoelectric focusing in an Ampholine pH gradient, as described previously [12].

3. Results and Discussion
Studies of the quality indicators of canned meat in accordance with regulatory documents are carried out on the average sample of the specimen [13]. When studying canned meat pieces using two-dimensional electrophoresis in accordance with generally accepted requirements (Figure 2), fragments of the main muscle and connective tissue proteins of beef were found in small quantities, and the pattern of the location of protein fractions of the studied meat system was preserved. However, the effect of overlapping proteins and a blurred image did not allow for a clear visualisation of the marker zones of the meat system, and therefore, it was decided to change the protocol for sample preparation of canned meat for studies by two-dimensional electrophoresis.

![Figure 2. Electrophoretogram of the average sample of canned food “Top grade stewed beef”. The marked ladder is the protein standard with different molecular weights, kDa](image)

In the manufacture of canned meat, rendered fat is used, in which there is practically no protein or raw beef fat, as after sterilisation and melting of fat, small pieces of connective tissue rind can contain up to 0.07% protein, which will not affect the results of electrophoretic studies if the fat component is removed. Therefore, a modified sample preparation protocol for canned meat containing meat pieces was developed before two-dimensional electrophoresis. The modified sample preparation was based on dividing the contents of a consumer package of the canned meat into its component parts to produce three samples and included the following sequence of operations.
Before opening, the can with contents was heated in a water bath for 20 minutes, then opened to 2/3 or 3/4 of the circumference. Then, the can was installed obliquely into a funnel and the liquid part of the canned food was drained into a beaker for 10-15 minutes, while every 5 minutes the can was carefully turned several times. Individual pieces of meat were removed with tweezers or a spoon and were finely chopped prior to electrophoresis. The drained liquid part of the canned food, broth with fat, was cooled in a beaker to 0°C-8°C. The solidified fat was removed from the broth surface. Two further samples were then produced – the liquid broth and a mixture of meat with broth.

The research results are shown in Figure 3. The most informative picture was obtained in the study of pieces of meat (Figure 3A) from the canned food. A large amount of proteins was detected in the entire molecular weight range of the polyacrylamide gel, including the main structural fractions of β-enolase, muscle creatine phosphokinase, phosphoglycerate kinase 1, glyceraldehyde-3-phosphate dehydrogenase, groups of troponins and myosin light chains.

![Electrophoretograms of canned food fractions from “Top grade stewed beef”](image)

**Figure 3.** Electrophoretograms of canned food fractions from “Top grade stewed beef”

Electrophoretic study of the broth (Figure 3B) from the canned meat revealed a large number of intensely coloured high-molecular-weight protein fractions with a mass of more than 50 kDa, probably collagen chains from connective tissue, as well as mitochondrial aconitase 2, heat shock proteins, desmin and actin fractions.

For the primary electrophoretic screening of the protein component of canned meat with meat pieces, it was optimal to use the sample option of broth together with meat pieces, with the fat component removed (Figure 3C). This method allowed the main protein components in a wide range of molecular weights to be identified.

4. **Conclusion**
The conditions for the separation of the protein component in a canned meat product with whole meat pieces were adapted by removing the fatty component of the product. For comparative analysis of high-molecular-weight proteins, it is optimal to study the protein fractions of the broth. To study the variations in tissue proteins, it is reasonable to study the solid component of canned meat pieces. However, to determine the total spectrum of proteins, electrophoretic study of the combined broth and meat pieces is recommended.

**References**

[1] Kowalska G, Pankiewicz U and Kowalski R 2020 Determination of the level of selected elements in canned meat and fish and risk assessment for consumer health *J. Anal. Methods Chem.* 2020
2148794 doi: 10.1155/2020/2148794

[2] Perestam A T, Fujisaki K K, Nava O and Hellberg R S 2017 Comparison of real-time PCR and ELISA-based methods for the detection of beef and pork in processed meat products Food Control 71 346–52 doi: 10.1016/j.foodcont.2016.07.017

[3] Krylova V B and Gustova T V 2017 Comparative dynamics of protein destruction in canned foods in sauce at different thermal treatment regimes and subsequent storage Theory and practice of meat processing 21 37–46 doi: 10.21323/2414-438X-2017-2-1-37-46

[4] Bello I, Simsek M, Olorunnisola S, Babiker F and Hammed A M 2021 Food Authentication and Traceability (Cambridge: Academic Press) chapter 9 pp 247–77

[5] Coorssen J R 2013 Top-down proteomics: 2D gels are an integral part of the process Farm animal proteomics 2013 (Wageningen: Wageningen Academic Publishers) pp 31–3

[6] Matsumoto H, Hanii H, Kurien B T and Komori N 2019 Two-Dimensional Gel Electrophoresis by Glass Tube-Based IEF and SDS-PAGE Electrophoretic Separation of Proteins (New York: Humana Press US) pp 107–13 doi: 10.1007/978-1-4939-8793-1_11

[7] Mora L, Gallego M and Toldrá F 2018 New approaches based on comparative proteomics for the assessment of food quality Curr. Opin. Food Sci. 22 22–7

[8] Chernukha I M, Fedulova L V, Vasilevskaya E R and Kotenkova E A 2017 Comparative study of biocorrective protein-peptide agent to improve quality and safety of livestock products Potr. S. J. F. Sci. 111 539–43 doi: 10.5219/590

[9] Gao J, Li T, Lu Z, Wang X, Zhao X and Ma Y 2019 Proteomic analyses of mammary glands provide insight into the immunity and metabolism pathways associated with clinical mastitis in meat sheep Animals 96 309 doi: 10.3390/ani906309

[10] Schilling M W, Suman S P, Zhang X, Nair M N, Desai M A, Cai K, Ciaramella M A and Allen P J 2017 Proteomic approach to characterize biochemistry of meat quality defects Meat Sci. 132 131–8 doi: 10.1016/j.meatsci.2017.04.018

[11] Zheng N Z, Zhu Z M, Xin Q W, Zhang Z H, Miao Z W, Li L, Zhang L L, Wang Z C and Huang Y F 2019 Differential protein profiles in duck meat during the early postmortem storage period Anim. Sci. J. 906 757–68

[12] Akhremko A, Fedulova L 2021 Comparative study of weaning pigs’ muscle proteins using two-dimensional electrophoresis Potr. S. J. F. Sci. 15 52–7

[13] Stojanović B, Vasilev D, Stojanović Z, Parunović N, Janković S, Stanojević S, Balaban M, Antić V 2021 Determination of sensory properties and levels of trace elements during storage of canned meat products J. Food Process. Preserv. 45 e15278 doi: 10.1111/jfpp.15278