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Biochemical changes in myofibrillar and sarcoplasmic meat proteins at different freezing rates

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Abstract

Freezing rate significantly affects the quality of frozen meat and its biochemical changes. The loss of water from the cellular structure, which, under normal conditions, acts as a mechanical barrier between protein chains, causes spontaneous interactions of proteins that result in their denaturation. In order to determine the content of myofibrillar and sarcoplasmic proteins in meat samples, the samples were frozen at rates of 0.40, 0.90, and 1.25 cm/h for a storage period of 15 days. Protein content was analyzed by capillary gel electrophoresis and high-pressure liquid chromatography. During the freezing process, the protein chains were found to be shortened so that myofibrillar proteins were between 20 and 50 kDa, whereas sarcoplasmic proteins were between 20 and 100 kDa at all the freezing rates mentioned. The results of the analysis also show that the total number of sarcoplasmic proteins (freezing rate 0.90 cm/h; 20 proteins) was lower than the total number of myofibrillar proteins (freezing rate 0.90 cm/h; 35 proteins), and the molecular weight for both types of proteins was lower 50 kDa. Further research should be directed towards extending storage time.

Keywords: meat, myofibrillar proteins, sarcoplasmic proteins, freezing rate.

1. INTRODUCTION

Proteins are basic constituents in the structure of cells and tissues of plant and animal organisms. They participate in many vital processes. They perform significant structural, catalytic, protective, transport, energy and hereditary functions (Pribiř, Dimić, & Šefer 1999). Freezing is one of the oldest and most commonly used food preservation techniques. Compared to other canning methods, frozen foods are best preserved for basic nutrients and labile food components. Proper freezing procedures allow food to be stored for an extended period of time (Savanović 2017).

Actin and myosin are major contractile proteins important for the formation of functional properties of meat. During freezing, myosin aggregation reactions occur causing an increase in meat hardness and a loss of water-binding ability (Jagica et al. 2007). Protein denaturation

reduces the amount of soluble proteins and free SH groups in meat (Chan, Omana, & Betti 2011; Wu, Pan, & Wang 2014). Denaturation, especially in the case of slow freezing, also involves the cleavage of actin and myosin bonds (Sučić, Cvrtila, Njari, Kozačinski, et al. 2010).

Coggins and Chamul (2004) state that the most significant physicochemical changes that occur during frozen food storage are lipid oxidation, protein denaturation, and protein discoloration. Accordingly, freezing of food products is performed at different rates, in order to examine the effect of freezing rate on the change in the native conformations of proteins. According to the speed of the ice zone, meat freezing procedures can be classified as slow (0.1–0.2 cm/h), rapid (0.5–3 cm/h), and extremely fast (5–10 cm/h and higher) (Mastanjević 2010).

Accordingly, freezing of food products was performed at different speeds (0.40; 0.90 and 1.25 cm/h) in order

to examine the effect of freezing rate on the change in the native conformation.

2. MATERIALS AND METHODS

2.1. Materials

The test was performed on pork backbone specimens taken from *M. Longissimus dorsi* pigs less than 8 months old and about 110–120 kg in average gross weight. After separating the muscles from the trunk, the pieces were cut into about 2.0 cm thick slices, packed in polythene bags and labeled. The samples were then frozen at different rates (0.40, 0.90, and 1,25 cm/h). Two groups of proteins were analyzed: myofibrillar and sarcoplasmic. Each group had 3 samples. The food samples were frozen at different temperatures and tested at the storage time of 15 days after freezing. The analyses performed were high-pressure liquid chromatography (HPLC) and capillary gel electrophoresis (CE).

2.2. Working methods

2.2.1. Capillary gel electrophoresis

Protein changes and behavior during freezing, storage and thawing of food products (qualitative and quantitative parameters) were monitored by capillary gel electrophoresis.

Capillary gel electrophoresis (CE) involves a set of analytical procedures and different operational and separation characteristics. This method involves separating and defining components from a mixture using an electric field established in the capillary. The technique is based on the principle that charged particles migrate in an electrolyte solution under the influence of a high potential electric field in a narrow capillary towards one of the electrodes.

Extraction of protein from meat (sarcoplasmic and myofibrillar proteins). Sarcoplasmic protein extracts were obtained according to the method of [Toldra, Rico, and Flores \(1993\)](#). Two grams of pork were homogenized with 20 ml of 0.03 mol/dm³ sodium phosphate buffer (pH = 7.40) at 4°C for 2 min using a homogenizer (UULTRA-TURRAX IKA T25 digital). The homogenate was centrifuged for ten minutes, 5000 rpm, at 4°C. The supernatant contained sarcoplasmic proteins. Myofibrillar proteins were extracted from the precipitate by homogenization with a solution containing 8 mol/dm³ urea and 1% β-mercaptoethanol at 4°C for 2 min using a homogenizer (ULTRA-TURRAX IKA T25 digital). The homogenate was re-centrifuged under the same conditions and a supernatant containing myofibrillar proteins was obtained.

Protein separation. Protein separation was performed by capillary gel electrophoresis (Agilent, CE 7100) using an SDS-MW Analysis Kit (Beckman Coulter), 10–225 kDa.

The contents of the SDS-MW Analysis Kit are:

- 2 capillaries 57 cm long, 50 μm I.D;
- SDS-MW Gel buffer – appropriate formulations;
- SDS Sample buffer – 100 mM Tris-HCl, pH 9.0/1% SDS;
- SDS Protein Standard (10 to 225 kDa);
- International Standard 10 kDa;
- 0.1 mol/dm³ HCl;
- 0.1 mol/dm³ NaOH.

SDS-MW standard preparation. To prepare the SDS MW standard, 10 μL MW standards were mixed with 85 μL of Sample buffer, 2 μL of international standard and 5 μL of 2β-mercaptoethanol. After gentle stirring, heating for 3 minutes at 100°C (Thermo-Shaker, Biosan, TS100) followed and the contents were cooled down to room temperature before injection.

Preparation of protein samples. Protein concentration in the extract was determined by the Lowry method. Samples of the extracted proteins were first filtered through 0.2 μm pore size filters and then dissolved in SDS buffer to a total volume of 95 μL. The protein concentration in the solution should be from 0.2 mg/mL to 2 mg/mL. Then, 2 μL of internal standard and 5 μL of 2β-mercaptoethanol were added. The contents were stirred gently and then heated for 3 minutes at 100°C (Thermo-Shaker, Biosan, TS100) to ensure denaturation of the proteins present, after which they were cooled down to room temperature before injection.

The detection wavelength was 220 nm with a bandwidth of 20 nm (without reference wavelength) and a response time of 1 second. For all reagents, 2 ml glass vials were used. The reagent fill volume was 1.2 ml, except for the vials filled with 1,6 ml water and three vials for reagent waste (0.1 mol/dm³ NaOH, 0.1 mol/dm³ HCl/water and SDS gel buffer) which contained 0.6 ml of water. SDS gel buffer was replaced after each sequence of 6 to 8 injections. The standard contained 7 proteins of known molecular weights (10 kDa, 20 kDa, 35 kDa, 50 kDa, 100 kDa, 150 kDa and 225 kDa). Proteins of known molecular weights (MW standards) were separated in the course of 30 minutes.

2.2.2. High-pressure liquid chromatography

Biochemical changes and protein behavior meat sample freezing at different freezing rates were monitored using high-pressure liquid chromatography (HPLC).

Table 1. Number of myofibrillar proteins in the tested pork samples stored for 15 days, (CE)

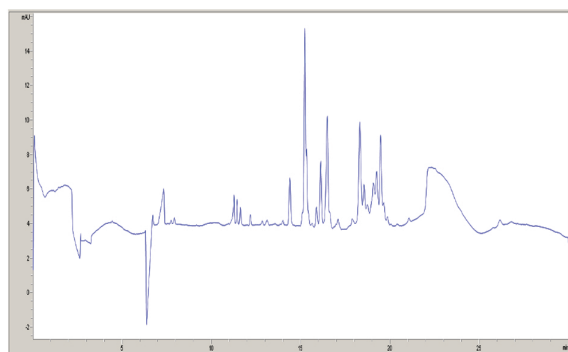
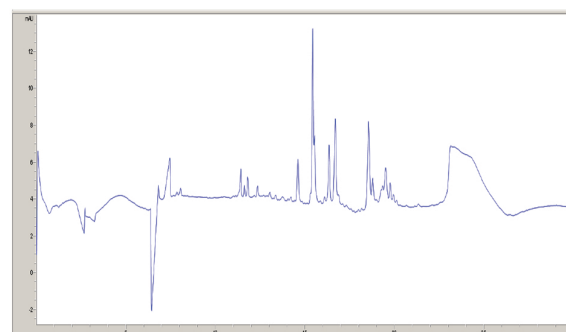
Sample tag	Freezing rate cm/h	<20 kDa	20-50 kDa	50-100 kDa	100-150 kDa	>150 kDa	In total	Concentration
9	0.40	21	13	–	–	–	34	100.0009
4	0.90	32	3	–	–	–	35	100.0000
10	1.25	13	13	–	–	–	26	100.0002
Fresh meat	/	21	13	8	3	3	48	99.9998

The main components of this system are the pump, the injector, the detector and the database. Mobile phase tanks and fraction collectors are also important. The HPLC pump delivers the mobile phase through the system, typically at a flow rate of 0.4–1 ml/min. Changing the loop allows the injection of different volumes. Although injection volumes of 10–100 μ L are typically used, both larger and smaller sample volumes can be achieved using special hardware.

The procedure for determining the protein content in meat using the HPLC method is as follows. After tempering the device and adjusting the operating parameters, the samples were filtered using a 0.45 μ m filter into the vials and analyzed for 25 minutes with a Postrun of 6 minutes.

3. RESULTS AND DISCUSSION

Biochemical changes in myofibrillar and sarcoplasmic proteins were determined by the analysis of samples

**Figure 1.** Electrophoregram, myofibrillar protein, freezing rate 0.40 cm/h, sample 9**Figure 2.** Electrophoregram, myofibrillar protein, freezing rate 0,90 cm/h, sample 4

frozen at different speeds (0.40, 0.90, and 1.25 cm/h) using capillary gel electrophoresis, as can be seen from the following electrophoregrams (Figures 1–3, myofibrillar and Figures 4–6, sarcoplasmic).

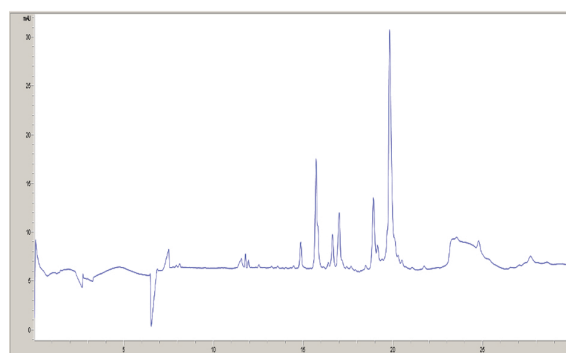
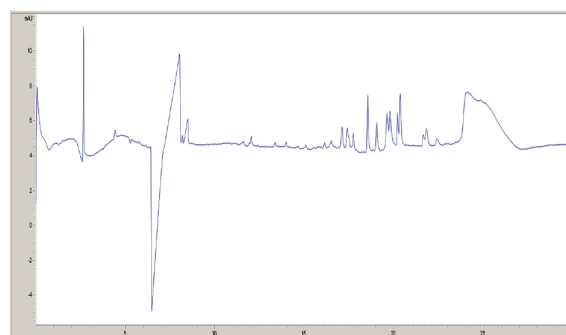
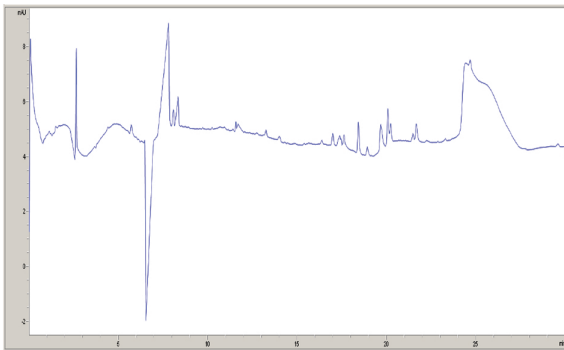
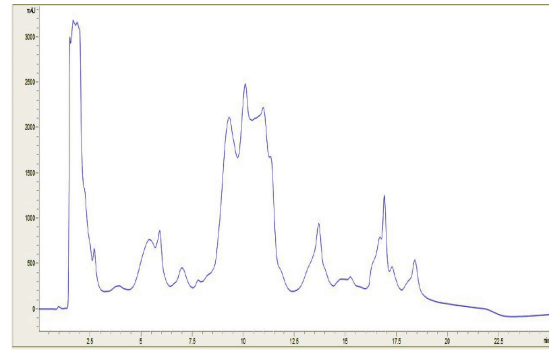
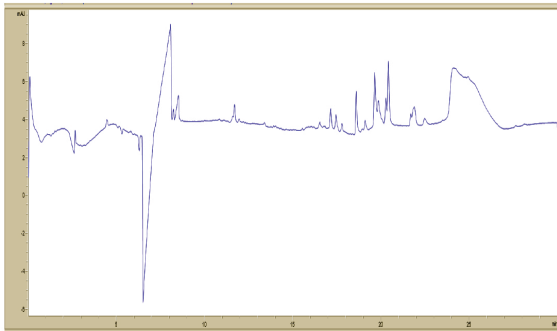
**Figure 3.** Electrophoregram, myofibrillar protein, freezing rate 1,25 cm/h, sample 10**Figure 4.** Electrophoregram, sarcoplasmic protein, freezing rate 0,40 cm/h, sample 9

Table 2. Number of sarcoplasmic proteins in the tested pork samples stored for 15 days, (CE)

Sample tag	Freezing rate cm/h	<20 kDa	20-50 kDa	50-100 kDa	100-150 kDa	>150 kDa	In total	Concentration
9	0.40	10	15	–	–	–	25	99.9997
4	0.90	9	11	–	–	–	20	100.0000
10	1.25	8	12	1	–	–	21	100.0001
Fresh meat	/	41	18	9	2	1	71	99.8867

**Figure 5.** Electrophoregram, sarcoplasmic protein, freezing rate 0,90 cm/h, sample 4**Figure 7.** Chromatogram, myofibrillar protein, freezing rate 0,40 cm/h, sample 9**Figure 6.** Electrophoregram, sarcoplasmic protein, freezing rate 1,25 cm/h, sample 10

Electrophoregrams (1, 2 and 3) for the analyzed meat samples frozen at 0.40, 0.90 and 1.25 cm/h show the difference in the content of myofibrillar proteins, with the lowest content at the highest freezing rate. It can also be observed that their size ranges from 20 to 50 kDa.

Electrophoregrams (4, 5 and 6) for frozen samples analyzed at 0.40, 0.90 and 1.25 cm/h show differences in the structure of sarcoplasmic proteins although their content obtained by HPLC analysis is small and indicates that they are less than 20 kDa.

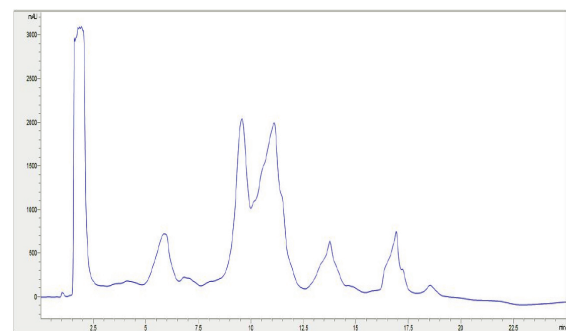
The following tables show the content of myofibrillar and sarcoplasmic proteins determined on the capillary gel electrophoresis apparatus in meat samples stored for 15 days.

Analyzing the content of myofibrillar proteins at different freezing rates (0.40, 0.90 and 1.25 cm/h), we find that their content is about 30% lower than that in fresh

meat (Table 1). Also, we can see that the freezing process had the effect of shortening their chains, so they were proteins between 20 and 50 kDa, while fresh meat contains proteins between 20 and 150 kDa (Table 1).

Although the number of sarcoplasmic proteins is smaller than myofibrillar proteins, their size is observed to be between 20 and 50 kDa for freezing rates of 0.40 and 0.90 cm/h, while for the freezing rate of 1.25 cm/h we also observe proteins up to 100 kDa. The number of sarcoplasmic proteins in fresh meat is about 70% higher than in frozen samples, regardless of the freezing rate.

Proteins identified in meat samples analyzed by high-pressure liquid chromatography (HPLC) apparatus at different freezing rates (0.40, 0.90, and 1.25 cm/h) are shown in the following chromatograms, myofibrillar (Figures 7–7) and sarcoplasmic (Figures 10–12).

**Figure 8.** Chromatogram, myofibrillar protein, freezing rate 0,90 cm/h, sample 4

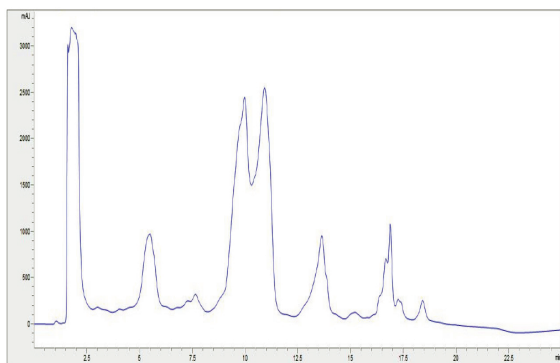


Figure 9. Chromatogram, myofibrillar protein, freezing rate 1,25 cm/h, sample 10

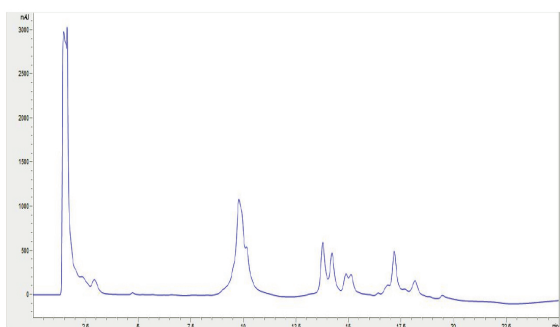


Figure 10. Chromatogram, sarcoplasmic protein, freezing rate 0,40 cm/h, sample 9

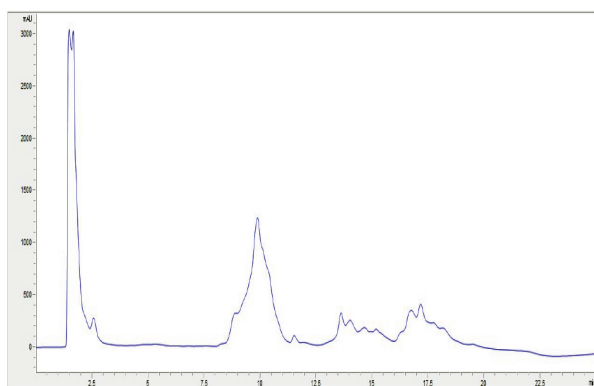


Figure 11. Chromatogram, sarcoplasmic protein, freezing rate 0,90 cm/h, sample 4

The following tables show the content of myofibrillar and sarcoplasmic proteins determined on a high-pressure liquid chromatography apparatus, in meat samples stored for 15 days.

The results of the analysis show that there are fewer total sarcoplasmic proteins than myofibrillar ones. In the group of myofibrillar proteins analyzed by high-pressure liquid chromatography, most proteins were isolated at a freezing rate of 0.40 cm/h, while the fewest sarcoplasmic

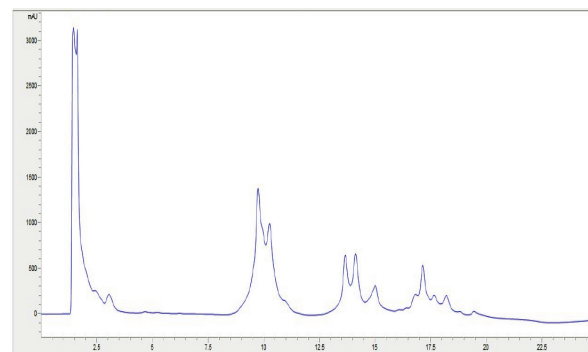


Figure 12. Chromatogram, sarcoplasmic protein, freezing rate 1,25 cm/h, sample 10

Table 3. Number of myofibrillar proteins in the tested pork samples stored for 15 days, (HPLC)

Sample tag	Freezing rate cm/h	<20 kDa	In total	Concentration
9	0.40	49	49	99.9998
4	0.90	30	30	100.0011
10	1.25	37	37	100.0000

proteins were isolated at a maximum freezing rate of 1.25 cm/h.

4. CONCLUSION

During the analyses of protein content and structure by capillary gel electrophoresis (CE) as well as by high-pressure liquid chromatography (HPLC), differences in the content of myofibrillar and sarcoplasmic proteins were observed.

During the freezing process, the protein chains were found to be shortened so that myofibrillar proteins were between 20 and 50 kDa, whereas sarcoplasmic proteins were between 20 and 100 kDa at all the freezing rates mentioned. In addition, the results of the analysis show that the total number of sarcoplasmic proteins (freezing rate 0.90 cm/h; 20 proteins) was lower than the total number of myofibrillar proteins (freezing rate 0.90 cm/h; 35 proteins), and the molecular weight for both types of proteins was lower 50 kDa.

The number of proteins of molecular weight between 50 and 150 kDa was 14 for myofibrillar and 12 for sar-

Table 4. Number of sarcoplasmic proteins in the tested pork samples stored for 15 days, (HPLC)

Sample tag	Freezing rate cm/h	<20 kDa	In total	Concentration
9	0.40	24	24	99.9563
4	0.90	34	34	99.9994
10	1.25	19	19	99.9999

coplasmic (Table 1 and Table 2). Proteins of this molecular weight do not appear after thawing, suggesting that they have been denaturated.

These changes indicate changes in protein composition during storage, so future research should aim to extend storage time at these freezing rates.

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