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EFFECTS OF TEMPERATURE AND METHOD OF HEAT TREATMENT ON MYOFIBRILLAR PROTEINS OF PORK

Article Highlights

- Thermal processing of meat cases proteins denaturation
- Meat was processed by roasting and cooking in water at atmospheric pressure
- By capillary electrophoresis was performed quantitative and qualitative proteins determination
- Proteins during heat treatment showed a drastic decline in solubility with increasing temperature
- Thermal processing by cooking had greater impact on proteins denatururation

Abstract

During the tests in this paper, meat processing was carried out at different temperatures in the range of 51 to 100 °C. The meat was processed by dry heat (roasting) and wet heat treatments (cooking) in water at atmospheric pressure. After heat treatment, myofibrillar proteins were extracted from solutions at constant ionic strength. Quantitative and qualitative determinations of protein fractions were performed by capillary electrophoresis. Myofibrillar proteins were also analized for fresh pork meat sample. Results obtained in fresh meat were compared with those recorded after roasting and cooking. In the fresh and thermally processed pork the following proteins were identified: myosin, light chain 3; myosin, light chain 2; troponin - C; troponin - I; myosin, light chain 1; tropomyosin; troponin - T; actin; desmin; α - actinin; C - protein; M - protein (M_β); M - protein (M_α); heavy meromyosin - HMM. For both methods of thermal processing, with increasing heat treatment temperature, concentration of soluble protein in the extract decreases rapidly after 51 °C. Cooking treatment had a more intense effect on the proteins change and denaturation than roasting.

Keywords: pork meat, cooking, roasting, protein denaturation, capillary electrop.

Protein denaturation in the most general form can be considered as a change in the structure of proteins, which occurs during thermal processing of meat. It is known that protein denaturation involves important changes in their structure, which may occur during thermal processing of meat. The temperature at which these changes occur is called denaturation temperature. In much of the literature, pertaining to the biochemistry of meat and studying the effect of heat treatment technology on the quality and characteristics of products, denaturation temperature was identified as a key moment in which the traits of meat rapidly changing. Besides heat, processes of protein denaturation are affected by other factors, such as pH and ionic strength of the solution. After protein denaturation in heat treated meat, intense reactions occur between protein chains (protein-protein interactions). The result of these changes is protein aggregation (coagulation or gelation) [1]. The above changes can be qualitatively and quantitatively determined by measuring changes in protein solubility and protein composition of the extracts using electrophoretic methods [2].

Heat treatment cause a change in myosin helix structure and surface hydrophobicity of light meromyosin LMM at pH 6 and 0.6 M KCl. LMM content in the form of a helix structure begins to fall at 30 °C and

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reaches a minimum at 70 °C. Along with that surface hydrophobicity increases in the samples heated to 65 °C, after which it begins to decline sharply. Reduction of surface hydrophobicity at higher temperatures suggests that a part of the hydrophobic residues in protein chains is involved in protein-protein interactions, during which aggregate networks are formed and the formation of gel occurs [3,4].

Protein-protein interactions in myofibrillar proteins already begin at a temperature range from 36 to 40 °C. This process precedes the first expansion and unfolding of long molecules of globular proteins at temperatures from 30 to 32 °C. More intensive myofibrillar protein gelation begins from 5 to 50 °C, as it can be seen by the intense increase in density of protein solutions. These processes are not affected by rigor state of the meat. All this reduces the solubility of newly formed aggregates of myofibrillar proteins at a given ionic strength [5–8].

Hydrophobicity of myofibrillar proteins, soluble in saline solution does not directly depend on the type of muscle, but of the way and type of protein-protein interactions and degree of gel formation. Accordingly, heat-induced denaturation of myofibrillar proteins in solution results in gel formation. Myosin has a high aggregation affinity in very small protein concentration of 0.5% [5,6,9,10].

If the myosin solution is heated, maximum gel strength, which is formed during that process, is reached at a temperature of 45 °C and pH 5.5 or 60 °C at pH 6 of the solution. If the myosin solution contains actin, gel strength increases at the same temperatures and pH values. Ionic strength and pH are important factors that determine whether the myosin is in monomeric form or in the form of connected filaments. At an ionic strength greater than 0.3 and neutral pH, myosin molecules are dispersed in solution as monomers that build coarse grid with large pores. At low ionic strength solutions, myosin molecules are linked into filaments, like naturally thick filaments in muscle. Such a gel consists of a fine and uniform mesh with small pores [7,11].

Myofibrillar protein gel formation during warming takes place in two steps and in two separate temperature intervals. The first part of the reaction occurs at temperatures between 30 and 50 °C, and second part of the reaction at temperatures above 50 °C. The first step involves aggregation process, where are protein--protein connections occur over myosin globular head. In the second step, changes occurring at the level of myosin helix structure, which leads to formation of networks, where hydrophobic groups have a primary role in creating linkages at the level of protein-protein interactions [12].

Myosin-myosin interactions do not occur in myosin solution, which has been heated for 30 min at 30 °C. When the same solution is heated for 30 min at a temperature of 35 °C, interactions between myosin molecules begin, where the solution becomes denser and has a prominent optical properties. Myosin molecules still dominate in their natural form in these conditions as well [13].

Heating at temperatures over 40 °C results in the change in myosin, which causes the loss of properties which the protein had in its natural form. Above 50 °C, the aggregation process is significantly accelerated and it is difficult to see individual myosin tails. Between 50 and 60 °C, if the myosin solution is heated for 30 min, leads to formation of large globular aggregates. At temperatures above 60 °C, individual myosin tails cannot be observed more in solution. By further heating (above 60 °C), helices are formed through the hydrophobic groups on the myosin chains [14].

Myofibrillar proteins build highly compact and stable gels, which have a high water holding capacity and good rheological properties. The conditions under which the resulting gel (ionic strength, pH, heating time, etc.) is formed affect the properties of the formed gel [1,7,14].

Unlike myofibrillar proteins with a high power of aggregation and creation of conglomerates with higher molecular weight even at very low concentration values in analyzed solutions, to start the process of sarcoplasmic protein aggregation, proteins must be present in solution at a concentration greater than 3% [15].

During heating process, meat proteins is denaturated, which causes a variety of changes in the structure of meat (destruction of cell membranes, tearing of muscle fibers, coagulation and gel formation of myofibrillar and sarcoplasmic proteins, breaking and dissolving protein of connective tissue, etc.). It is known that there are differences in the connective tissue and muscle structure that may affect the degree of the above changes and temperature at which these changes occur [16].

To the best of our knowledge, there are no available literature data on application of this electrophoretic technique for evaluation of changes of meat protein in the thermally treated meat sample so far. Here, for the first time, a lab-on-a-chip technique was applied to detection of protein changes in both cases of heat treatment.The main objective of this paper was to prove that different temperatures in tempera-

ture range from 51 to 100 °C and various heat treatment processes (roasting and cooking) can differently affect the state of myofibrillar proteins during heat treatment.

EXPERIMENTAL

Samples and sample preparation

The study was conducted on the pork meat, reared on a modern farm in Bosnia and Herzegovina. Pigs were between eleven and twelve months of age and had an average gross weight of about 130–140 kg. The animals were bled under identical conditions. After cooling at 4 °C for 24 h, *Longissimus dorsi* from six carcasses was removed. The muscles were frozen at -20 °C, cut into slices of 2.0 cm of thickness.After labeling the samples were packed in polyethylene bags, refrozen at a temperature of -30 °C and kept at that temperature until the moment of testing. At the time of analysing the samples were thawed overnight in a refrigerator at a temperature of 4-5 °C.

Heat treatment of samples

Thawed samples were subjected to wet and dry heat treatment. Dry heat treatment was carried out by roasting (slices thickness of 2.0 cm) in oven type "Elit" 3 kW. The samples were heated to achieve desired temperature in the center of sample in range from 51 to 100 °C. The air temperature in the oven during all the experiment was 163±2 °C. The temperature in the oven and the temperature in the center of the sample were continuously monitored using a dual-channel thermocouple "TESTO" and "HANNA" HI 98810.

Wet heat treatment was carried out in a water bath. Before putting in water, samples were wrapped in thermosetting plastic bags in absence of air, and then heated to achieve the desired temperature in the center of the sample. The temperature was continuously monitored using a dual-channel thermocouple, "TESTO" and "HANNA" HI 98810. In case of wet treatment, thermocouple probe was placed in the center of the sample, and then wrapped in a plastic bag and tied at the top of the bag in the absence of air.

Determination of protein status

Sample preparation and protein extraction

Extraction was carried out for samples of fresh and heated meat. The sample mass of 0.5 g was taken for extraction and mixed with 5 cm^3 extraction buffer (8 M urea and 0.6 M NaCl to volume ratio 1:10), then homogenized using an Ultra turrax IKA T25 homogenizer for 30 s at 10000 rpm and left to stand for 12 h at 4 °C for protein extraction. During extraction, samples were continuously mixed with magnetic stirrers [16].

After extraction, the solution was centrifuged in an Eppendorf 5415C centrifuge at 16000*g* for a period of 10 min. After centrifugation, the supernatant was applied to the chip for capillary gel electrophoresis (Protein KIT 230 kDa, Agilent) by micropipette.

Qualitative and quantitative determination of protein status

Separation of proteins in prepared samples was performed by electrophoresis on a chip device Agilent 2100 bioanalyzer (Agilent Tehnologies, Santa Clara, CA) by using Protein 230 Plus Lab Chip kit.

The results were analyzed by the Agilent 2100 bioanalyzer software and shown in simulated images of gels (as scanned SDS-PAGE gels). Separation of proteins was carried out on the basis of their relative mobility in the gel. The specific proteins are qualitative separated by molecular weight of protein fractions, and quantified by the concentration of each of the identified protein fractions.

Meat samples which were roasted to end-temperatures from 51 to 100 °C, were marked as 51 °C (1), 61 °C (3), 71 °C (5), 81 °C (7), 91 °C (9) and 100 °C (11), shown in Figure 1. Meat samples which were cooked to end-temperatures from 51 to 100 °C, were marked as 51 °C (2), 61 °C (4), 71 °C (6), 81 °C (8), 91 °C (10) and 100 °C (12), shown in Figure 2. As a control a sample of fresh meat (sample that is not thermally processed) was included in Figures 1 and 2 marked as (0).

Statisitcs and data analysis

The experiments were a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's multiple range test at $p < 0.05$ significance level.

RESULTS AND DISCUSSION

The research results are presented in Figures 1 and 2, which show protein gel images separated by electrophoresis for fractions of fresh meat and meat processed by either roasting or cooking. Figures 3 and 4 show the change in the concentration of total soluble protein and number of bands for both methods of thermal processing. Tables 1 and 2 represent the mean change in the concentration of individual proteins identified from samples of pork meat, processed by roasting and cooking, at different temperatures in the middle of the sample in chosen temperature range from 51 to 100 °C.

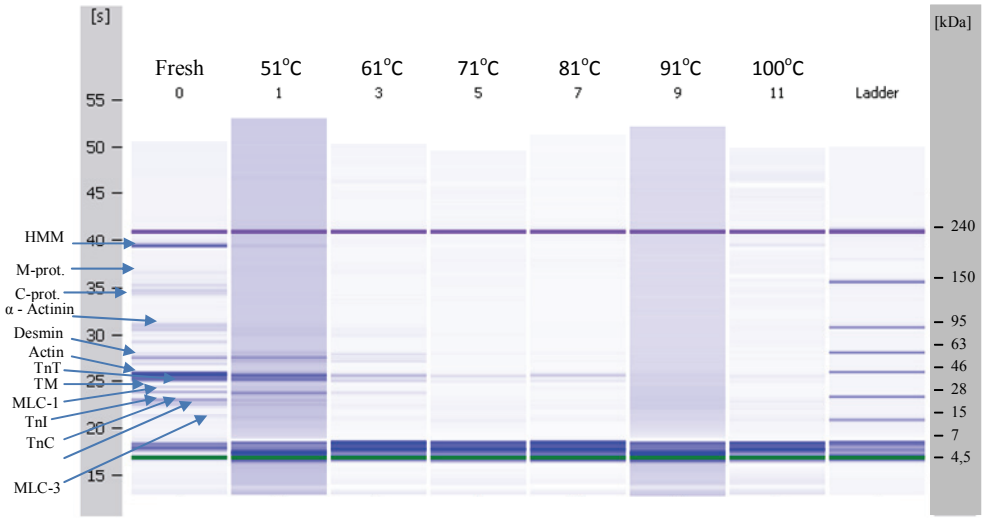

Figure 1. Gel images of electrophoresis separated meat protein fractions processed by roasting heat treatment.

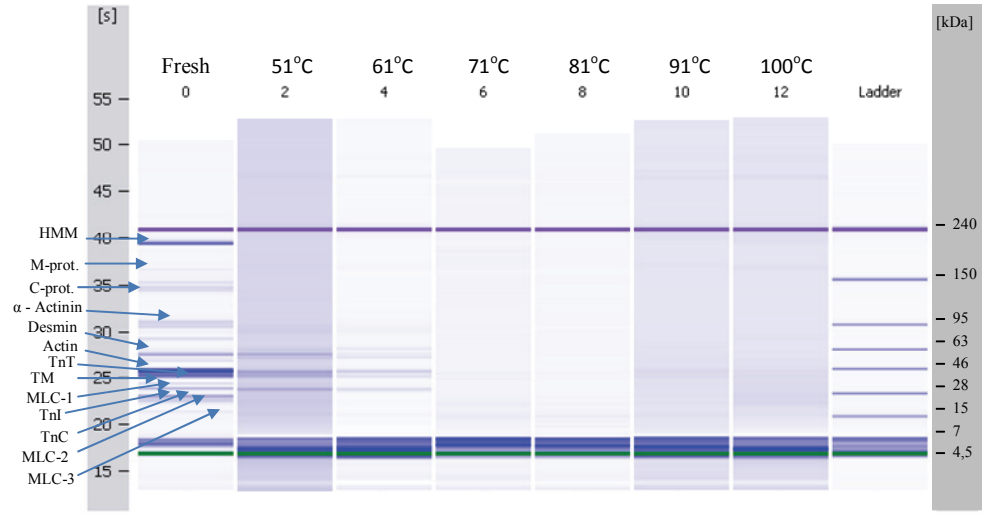

Figure 2. Gel images of electrophoresis separated meat protein fractions processed by cooking heat treatment.

Qualitative protein determination

Figures 1 and 2 separately show results for samples processed by roasting and cooking, respectively.

The gel images, obtained by separating the proteins from meat samples treated with both heat treatment processes, differ from each other according to the number of bands and according to their concentration. All gel images of thermally processed samples are quite different compared to the gel image samples of fresh meat. The total amount of soluble proteins decreased in the whole molecular range and at all preparation temperatures, regardless of the method, compared to fresh meat (Figures 1 and 2).

Dynamics of qualitative changes in protein from fresh meat to heat treated meat shows that in the range from 51 to 61 °C there was a disappearance of bands with molecular weights from 130 to 346 kDa. Thermal treatment in the temperature range from 61 to 91 °C leads to disappearance of bands with molecular weights (60–130 kDa) and at the end, from 91 to 100 °C, only protein bands with molecular weight (30–60 kDa) remains in the extract.

In fresh meat, 54 bands were identified and this increased in roasted meat until 61 °C where 93 bands were identified. Above 61 °C, the number of bands decreased and at 100 °C 59 bands were identified (Figure 3). In cooked samples the total number of bands increased to the temperature of 61 °C (93 bands), then droped at 71 °C to 54 bands. From 71 to 91 °C, the number of bands increased to a value of 131, and finally at 100 °C 95 bands were identified (Figure 4). In the temperature range from 61 to 81 °C, when changes in the myofibril are most intense, the number of bands are approximately equal, and at the

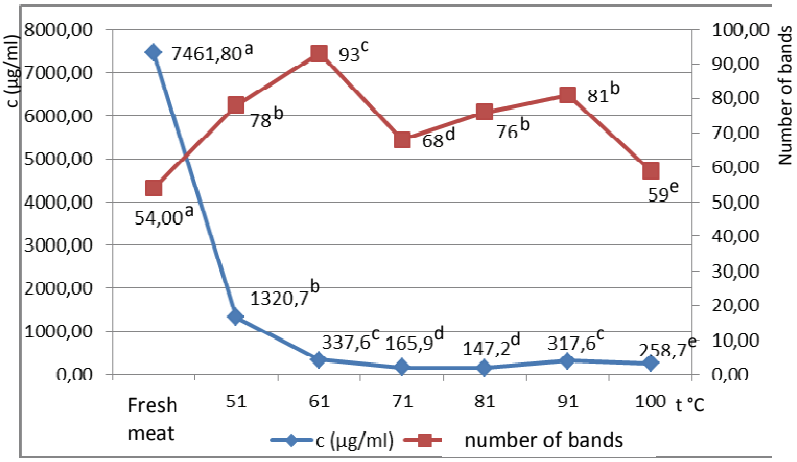

Figure 3. Changes in total protein concentration and the number of bands during roasting heat treatment; a, b, c..., e - different letters in superscript indicate statistical significance ($P \leq 0.05$).

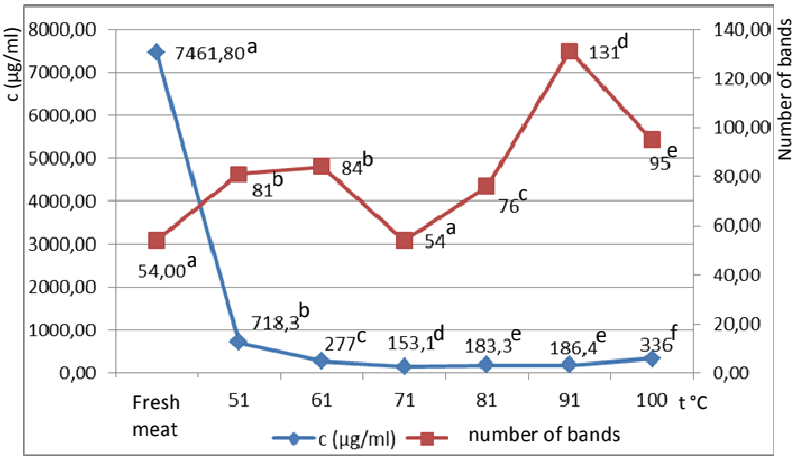

Figure 4. Changes in total protein concentration and the number of bands during cooking heat treatment; a, b, c..., e - different letters in superscript indicate statistical significance ($P \leq 0.05$).

temperature of 81 °C identical numbers of bands (76) for both methods of thermal processing are seen (Figures 3 and 4). Compared to the previous temperature intervals, the methods of thermal processing have significantly different ($P \leq 0.05$) effects on the changes and breakdown of myofibrils helix structure in the temperature interval from 91 °C to 100 °C, as can be seen from the significantly different number of bands ($P \leq 0.05$).

Different types of heat treatment and age of the animals from which it is extracted have different effects on the properties and gelation of collagen, which begins above 80 °C [1,11,17]. The results presented in this paper are largely consistent with previous results.

Myosin HMM is first band, that disappears dramatically on 51 °C, at samples processed by cooking, while at roasted samples can occur in decreased concentrations and in 61 °C [1,18]. The research results that were obtained in this paper are in partial agreement with previous results concerning to the solubility of myosin HMM. This is due to the different experimental conditions in terms of height of ambient temperature, the heating rate (51 to 100 °C) and heat diffusion during the heat treatment. In addition, there is a significant impact of the extraction buffers ionic strength.

Between 65 and 87 °C many changes are taking place in the myofibrillar proteins, which causes their coagulation, decreases the solubility and disappearance of the bands. Great contribution to protein heat denaturation, appearance and layout of bands has hydrolysis of proteins. [19]. As can be seen from Figures 1 and 2 in the fresh and thermally processed pork the following proteins were identified: myosin, light chain 3; myosin, light chain 2; troponin - C; troponin - I; myosin, light chain 1; tropomyosin; troponin -

Table 1. Mean values of individual differences in the concentration of proteins (c in μg/cm³ ±SD) identified from samples of pork meat processed by dry heat (roasting) process at different temperatures in the middle of the sample; a, b, c..., e - different letters in superscript indicate statistical significance (P ≤ 0.05)

Protein name	Desired temperature in the centre of the samples, t_c / °C						
	Fresh	51	61	71	81	91	100
MLC 3	6.4a±0.98	45.2b±2.34	11.4c±0.54	22.3d±1.75	9.0c±0.69	16.7e±1.89	24.4d±1.93
MLC 2	71.7a±2.43	22.4b±1.90	0.0c±0.00	2.8d±0.54	2.8d±0.94	8.6e±0.94	5.8f±0.89
Troponin - C	28.6a±1.42	31.3b±2.12	5.9c±0.96	90.9d±2.98	5.2c±0.51	14.9e±0.87	13.1e±1.11
Troponin - I	244.4a±5.90	57.2b±3.56	8.7c±0.87	8.4c±0.74	4.4d±0.49	21.1e±1.73	9.2c±0.23
MLC 1	596.1a±23.1	0.65b±0.11	4.8c±0.25	7.3d±0.39	5.3c±0.73	26.5e±1.84	17.4f±0.98
Tropomyosin	115.1a±6.89	0.0b±0.00	0.0b±0.00	0.0b±0.00	0.0b±0.00	0.0b±0.00	0.0b±0.00
Troponin - T	1393.6a±42.98	267.7b±23.56	50.7c±3.46	11.8d±0.68	23.7e±1.79	50.1c±2.85	8.0f±0.56
Actin	2419.5a±87.93	348.8b±37.91	98.0c±4.28	37.6d±1.89	67.9e±3.29	29.2f±1.94	25.6f±1.59
Desmin	128.8a±21.69	39.7b±2.97	21.1c±1.89	0.6d±0.09	2.2e±0.12	1.4e±0.08	2.8e±0.34
α - Actinin	158.5a±31.32	2.7b±0.23	0.2c±0.01	0.4c±0.08	0.4c±0.01	0.5c±0.02	1.3c±0.25
C - protein	141.1a±12.85	0.0b±0.00	0.0b±0.00	0.6b±0.04	0.0b±0.00	1.7c±0.14	5.5d±0.71
M - Protein (M_β)	8.7a±1.12	0.3b±0.02	1.5c±0.13	2.8d±0.35	4.5e±0.29	0.7b±0.09	5.6e±0.69
M - Protein(M_α)	0.7a±0.05	0.5a±0.01	0.2a±0.01	2.4b±0.12	1.1c±0.11	0.2a±0.01	3.5d±0.92
Heavy meromyosin - HMM	807.9a±32.67	0.2b±0.01	0.1b±0.00	4.3c±0.68	0.7b±0.03	0.0b±0.00	1.5d±0.10

Table 2. Mean values of individual differences in the concentration of proteins (c in μg/cm³ ±SD) identified from samples of pork meat processed by wet heat (cooking) process at different temperatures in the middle of the sample; a, b, c..., e - different letters in superscript indicate statistical significance (P ≤ 0.05)

Protein name	Desired temperature in the centre of the samples, t_c / °C						
	Fresh	51	61	71	81	91	100
MLC 3	6.4a±0.83	28.5b±2.23	6.4a±0.67	39.9c±2.94	22.2d±1.58	12.5e±0.84	9.4f±0.49
MLC 2	71.7a±3.67	16.2b±1.56	0.0c±0.00	7.4d±0.49	24.8e±2.91	1.4c±0.09	8.2d±0.72
Troponin - C	28.6a±1.98	12.1b±1.23	0.9c±0.02	12.1b±0.99	3.5d±0.28	7.6e±0.38	42.0f±3.91
Troponin - I	244.4a±19.87	47.5b±3.12	1.9c±0.13	8.4d±0.91	5.3e±0.42	3.7e±0.41	26.6f±2.94
MLC 1	596.1a±29.34	20.9b±1.82	0.0c±0.00	7.6d±0.37	6.0d±0.31	6.0d±0.49	88.8e±3.67
Tropomyosin	115.1a±19.81	0.0b±0.00	0.0b±0.00	0.0b±0.00	0.0b±0.00	0.0b±0.00	0.0b±0.00
Troponin - T	1393.6a±37.91	108.5b±27.43	32.4c±1.54	7.5d±0.84	0.6e±0.00	32.9c±2.94	45.3f±3.94
Actin	2419.5a±42.98	118.6b±12.85	61.3c±2.82	8.1d±0.81	7.1d±0.83	28.0e±1.99	28.0f±1.39
Desmin	128.8a±12.81	24.1b±2.13	21.0b±1.86	0.1c±0.01	1.3c±0.04	0.0c±0.00	0.0c±0.00
α - Actinin	158.5a±13.16	0.6b±0.01	1.4b±0.11	0.2b±0.01	1.5b±0.08	0.5b±0.02	0.5b±0.01
C - protein	141.1a±18.47	0.1b±0.01	0.1b±0.01	1.3b±0.11	0.3b±0.02	0.6b±0.01	0.4b±0.02
M - Protein (M_β)	8.7a±1.82	1.0b±0.09	0.6b±0.02	3.3c±0.22	3.1c±0.11	1.4b±0.07	0.3b±0.01
M - Protein(M_α)	0.7a±0.04	0.3a±0.01	0.6a±0.01	1.4b±0.37	1.7b±0.12	0.0a±0.00	0.0a±0.00
Heavy meromyosin - HMM	807.9a±31.32	0.2b±0.01	1.0b±0.07	4.6c±0.89	1.1b±0.09	0.8b±0.06	0.0b±0.00

T; actin; desmin; α – actinin; C – protein; M – protein (M_β); M – protein (M_α); heavy meromyosin – HMM.

Quantitative protein determination

Changes in total protein concentration in extracts obtained from samples of fresh meat and meat samples thermally processed by cooking and roasting at temperatures from 51 to 100 °C, are shown in Figures 3 and 4.

The total concentration of soluble myofibrillar proteins in the samples of meat extract processed by roasting in the observed temperature range shows a trend of rapid reduction from 51 to 81 °C; then, it shows a slight increase in the temperature interval from 81 to 91 °C; finally, there is a re-reduction of concentration of soluble protein from 91 to 100 °C (Figure 3). As with roasting, the total concentration of myofibrillar proteins in the meat extract treated by

cooking tends to decrease rapidly with increasing temperature in the center of the sample from 51 to 71 °C; after that, further increase in temperature retains an approximately constant value. Up to 100 °C, the value is higher compared to the previous interval (Figure 4).

Tables 1 and 2 show the changes in the concentration of individual proteins identified by both methods of thermal processing. There are three intense bands in the extract from fresh samples (Tables 1 and 2): heavy meromyosin-HMM (~200 kDa), concentration 807.9±32.67 μg/cm^3, actin (~44 kDa), concentration 2419.5±87.93 μg/ cm^3 and troponin - T (~37 kDa), concentration 1393.6±42.98 μg/cm^3. In SDS-PAGE electrophoresis profiles of beef extract many authors have detected all three bands, with the greatest concentration of myosin and actin [1,20-22].

As can be seen from Tables 1 and 2 for both methods of thermal processing, with increasing heat treatment temperature, the concentration of soluble protein in the extract decreases rapidly after 51 °C. The only exception was myosin, light chain 3 whose concentration increases, from 6.4±0.98 μg/cm^3 in the extract of fresh meat, to 9.4±0.49 μg/cm^3 after reaching 51 °C in the center of the sample treated by cooking. By dry heat treatment (roasting), after reaching 51 °C concentration of myosin, light chain 3 was 24.4±1.93 μg/cm^3. After 51 °C all identified proteins larger than 100 kDa, such as α - actinin, C - protein, M - proteins and heavy meromyosin - HMM show a major decline in intensity. This is characteristic for both methods of thermal processing. In addition to these proteins with higher molecular mass, only tropomyosin with molecular weight less than actin disappears completely after 51 °C.

With increasing temperature during heat treatment, solubility of myofibrillar proteins decreases sharply, especially those with higher molecular mass. Exceptions are small proteins with molecular masses less than 50 kDa, which may in some cases deviate from this rule at temperature in the center of 50 °C [8,18,23]. The results in this paper are consistent with results of previous research.

With further increase of temperature up to 100 °C, the tropomyosin band does not appear, and the concentration of proteins with larger molecular weight appear only in traces, on the order of 10^{-6} g/cm^3 at the ionic strength of the solution used for extraction. The presence of these protein fractions traces is a result of their denaturation and confirmational changes in observed temperature range from 51 to 100 °C. The high thermal stability shows actin and desmin, interfilamental constituent of myofibrils. Their concentration in roasting heat treatment slightly increases above 81 °C in relation to the interval of myofibrils collapse (Table 1). Desmin vanishes for both processes of heat treatment after 71 °C and there is still in a trace, and actin appears throughout the observed temperature range, although with decreased concentration from 51 to 100 °C. This is consistent with studies that suggest that desmin and actin have high stability with increasing temperature during heat treatment [23].

The decrease in concentration is larger for the cooking samples. Characteristic for all identified proteins with lower molecular weight, maximum concentration changes occur in the temperature range from 61 to 81 °C. It is the interval during which decomposition of myofibrils appears as a consequence of this conformational change. All identified proteins with lower molecular weight than actin, except tropomyosin, show good thermal stability and also appear on 100 °C. At heat processing by cooking, this increase of concentration is higher (Table 2) than the heat treatment by roasting, but happens above 91 °C.

In contrast to the decrease in protein fractions in the present experiment, amino acid fraction in sample extracts increased with increasing heat treatment temperature [24,25].

Thus, above 51 °C, proteins with molecular weight greater than 100 kDa, such as α - actinin, C - protein, M - proteins and heavy meromyosin - HMM, did not get more soluble in any of the heat treatments. In both procedures of heat treatment, characteristic is that a tropomyosin disappears at 51 °C and above. On average, at heat treament above 61 °C, the extract in quantitative terms contains mainly desmin, actin, troponin - T, troponin - I, *i.e.*, proteins with lower molecular weight. This can be explained by the decomposition of myofibrillar structure and numerous conformational changes that occur at the level of protein-protein interactions. During heat treatment, products of proteins hydrolysis are also appearing in extracts, resulting in an increase of light proteins fraction (≤ 30 kDa), as the temperature rises. This is more evident in the process of heat treatment by cooking, because of more uniform heat diffusion due to lower temperature gradient and prolonged residence time to achieve the targeted temperature [25,26].

As could be seen from the increase in temperature during heat treatment, those proteins that are disappearing from the extract solution, which are mainly proteins of larger molecular weight, such as, *e.g.*, heavy meromyosin - HMM, first enter into a protein-protein interactions and form aggregates with larger molecular weights. For extraction solution that

was used in this study, these aggregates have low solubility and appear in trace. As a continuation of this research it would be useful to identify hardly soluble aggregates of molecular weight greater than 300 kDa. To do so, it is necessary to use the increased ionic strength solutions in order to increase the solubility of aggregates with higher molecular weight. In this way, it could be possile to detect the proteins and make a more accurate identification. Also, it should be noted that in this paper is used the protein detection kit whose detection range of molecular weights is ranged from 4.5-240 kDa. Thus, in future studies, a recommended protein detection kit with upper molecular weight minimum of 400 kDa could also be used.

CONCLUSION

The results in this paper prove that different heat treatment techniques have different effects on the state of proteins at the same temperature in the center of sample. As presented, electrophoretic examination of state of proteins during heat treatment showed a drastic decline in the solubility of protein fractions with increasing temperature in the center of the sample during heat treatment. A significant faster decrease ($P < 0.05$) in concentrations of soluble proteins was observed in cooked compared with roasted samples. This result is a consequence of more uniform heat diffusion in samples processed by cooking heat treatment, and a longer residence time to achieve the desired temperature in the center of the sample. Potential application of results that were obtained in this study lies broadly in industrial conditions with respect to the selected interval of the heat treatment, which is usually the most common used. Based on this study, it can be concluded that thermal treatment by cooking has more intensified impact of changes in protein structure.

Nomenclature

HMM – Heavy meromyosin;
TnT – Troponin – T;
TM – Tropomyosin;
TnI – Troponin – I;
TnC – Troponin – C;
MLC-1 – Myosin, light chain 1;
MLC-2 – Myosin, light chain 2;
MLC-3 – Myosin, light chain 3

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NAUČNI RAD

UTICAJ TEMPERATURE I METODE TOPLOTNE OBRADE NA STANJE MIOFIBRILARNIH PROTEINA MESA SVINJA

Tokom ispitivanja u ovom radu, toplotna obrada mesa je provedena na različitim temperaturama u temperaturnom intervalu od 51°C do 100°C. Meso je obrađeno suvom toplotnom obradom (pečenjem) i vlažnom toplotnom obradom (kuvanjem) u vodi na atmosferskom pritisku. Nakon toplotne obrade, miofibrilarni proteini su ekstrahovani slanim rastvorima konstantne jonske jačine. Kvantitativna i kvalitativna određivanja proteinskih frakcija izvršena su upotrebom kapilarne elektroforeze. Miofibrilarne proteinske frakcije analizirane su i iz uzorka svježeg mesa. Dobijeni rezulteti za svježe meso su poređeni sa rezultatima za uzorke obrađene toplotnim obradama kuvanje i pečenje. Kako u svježem, tako i u toplotno obrađenom mesu svinja, identifikovani su sledeći proteini: miozin, laki lanac 3; miozin, laki lanac 2; troponin - C; troponin - I; miozin, laki lanac 1; tropomiozin; troponin - T; aktin; dezmin; α - aktinin; C - protein; M - protein (M_β); M - protein (M_α); teški meromiozin - HMM. Za obe metoda toplotne obrade, sa povećanjem temperature tokom toplotne obrade, koncentracija miofibrilarnih proteina u ekstraktu značajno opada posle 51 °C. Toplotna obrada kuvanjem imala je značajnji uticaj na promjene i denaturaciju proteina, posmatrano u odnosu na toplotnu obradu pečenjem.

Ključne reči: meso svinja, kuvanje, pečenje, denaturacija proteina, kapilarna elektroforeza.