

ANALYSIS OF CHANGES OF THE HISTOLOGICAL STRUCTURE OF HAM MUSCLES AS AFFECTED BY CURING AND THERMAL TREATMENT*

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Key words: muscle structure, curing, plasticization, pasteurisation

The objective of the presented study was to assess the dynamics of changes of the histological parameters of the following two pork muscles: the *semimembranosus* muscle (*musculus semimembranosus*) and the *quadriceps* muscle of the thigh (*musculus quadriceps femoris*) under the influence of curing, massaging and pasteurisation processes with the assistance of the computer image analysis. The analysis of the muscle structure was conducted on the basis of: the surface and circumference of muscle fibres, Feret's H and V diameter, percentage proportion of muscle fibres and their quantity in the analyzed field of view. The fibre shape was determined on the basis of the H/V ratio. It was found that the *quadriceps* muscle of the thigh exhibited greater dynamics of changes of the structure elements in comparison with the *semimembranosus* muscle under the influence of injection and plasticizing processes. The injection of ham muscles resulted in the loosening of muscle fibres, rounding of their contours as well as in the increase of their cross section area. The pasteurisation process failed to cause changes in the structure of the *quadriceps* muscle of the thigh, whereas in the *semimembranosus* muscle, pasteurisation increased the cross section area and circumference of muscle fibres and their percentage proportion in the examined field of view in comparison with the massaged muscle. The applied pasteurisation process was found to even out cell dimensions and to decrease inter-cellular spaces in both of the examined muscles.

INTRODUCTION

One of the most important features of meat products influencing their quality as well as consumer acceptability is the product texture understood as a set of traits resulting from elements of its structure, their mutual organization and interactions [Dolata, 1993; Drobisz-Kopydłowska, 1997]. Muscle fibres constitute one of many muscle structure elements associated with ham quality [Aguilera, 2005]. Their thickness, quantity and type exert a significant influence on meat texture [Kłosowska, 1973; Swatland, 1985]. Some researchers maintain that muscles characterised by muscle fibres of greater diameter are tougher [Lachowicz, 2003].

The product final quality depends not only on the muscle structure used as the raw material for its production but also on a number of such technological processes as: injection with curing brine, plasticization and pasteurisation. Massaging, in other words, the process of mechanical treatment of the muscle tissue, which consists in the rubbing and/or thumping of meat pieces against one another and the walls of the massaging machine, results in the destruction of muscle structure. Structural changes within cell membranes and entire muscle fibres become more pronounced with the duration of the massaging treatment [Katsaras & Budras, 1993; Müller, 1989; Theno *et al.*, 1978; Siegel *et al.*, 1978]. The plasticization process facilitates brine absorption, enhances protein extraction and, most importantly, assists in bringing pro-

teins – mainly myofibrillar ones – to the surface of massaged muscles. Acting together with other chemical components of the brine and muscle structural constituents they form the so called 'binder' which is decisive in binding together pieces of meat during the thermal treatment [Müller, 1989; Tyszkiewicz, 1995; Xaragaño *et al.*, 1998; Gajowiecki *et al.*, 2001]. Therefore, it can be said that during the process of massaging the muscle tissue is affected by forces of underpressure and high pressure which change meat properties following alterations occurring in the protein substance of the meat, primarily, in the protein myofibrillar fraction [Stanley *et al.*, 1994; Tyszkiewicz & Jakubiec-Puka, 1995; Dolatowski, 1999]. The applied plasticization process should allow maximal swelling of proteins, lead to the dissociation or disruption of the actomyosin complex and open up access of brine to intracellular myofibrillar proteins [Tyszkiewicz, 1991]. By accelerating the break-up of the protein structure in the muscle tissue, it gives the tissue the so-called mechanical tenderization on maceration [Stanley *et al.*, 1994]. It can be assumed from literature data on the subject that muscles from slaughtered animals show differences in their texture [Shackelford *et al.*, 1995] and structure [Wiklund *et al.*, 1998] as well as in their susceptibility to the plasticization process [Motycka & Bechtel, 1983; Shackelford *et al.*, 1989]. Therefore, it can be assumed that each type of muscles requires different massaging parameters and their most common measure is the massaging time [Lachowicz *et al.*, 2003]. The dynamics of the

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histological changes taking place during the process of plasticization is influenced, primarily, by the construction of the massaging machine, duration of the treatment and the programmed massaging cycle. As the mechanical destruction of the muscle natural structure progresses, the meat plasticity and water holding capacity as well as the dynamics of the collagen thermal hydrolysis during the thermal treatment also increase. According to Siegel *et al.* [1978], destructive changes in the muscle tissue caused by the massaging process proceed faster in the presence of salt and phosphates. However, excessive massaging may lead to the destruction of the meat tissue structure resulting in the production of large quantities of muscle fibre pulp on the surface of the raw material. This is connected with the extraction of large quantities of proteins whose content in the brine drip in the 12th hour of massaging may reach 12%. On the other hand, insufficient massaging time or inadequate intensity of unit loading fail to improve the properties of the raw material and do not result in the desirable tenderness and juiciness of the finished product. The massaging process conducted optimally leads to such a degree of muscle protein degradation that they increase their hydration and water holding capacity during thermal processing, have their outer structures damaged slightly and the protein myofibrils released from them bind the individual pieces into one, uniform meat block which preserves its fibrous structure. That is why the time of the effective massaging should be adjusted to the type of the applied raw material, programmed massaging cycle and the employed equipment, *i.e.* its construction, size and operational parameters [Dolatowski & Stasiak, 1996].

The objective of the presented study was to assess the dynamics of changes of histological parameters of two pork muscles: the *semimembranosus* muscle (*m. semimembranosus*) and the *quadriceps* muscle of the thigh (*m. quadriceps femoris*) under the influence of curing, massaging and pasteurisation processes with the assistance of the computer image analysis.

MATERIAL AND METHODS

Experimental material. The experimental material comprised muscles making up the pork ham: the *semimembranosus* muscle (*m. semimembranosus*) and the *quadriceps* muscle of the thigh (*m. quadriceps femoris*). After cutting the muscles out from the carcass, they were divided into two parts: one part was treated as the control, while the other was subjected to injection with the curing brine at the temperature of +4°C with the aid of a multi-needle injector. The average pH value of the brine used for the injection was 6.75. The applied injection with the curing brine was 40% in relation to the muscle weight. Each of the injected samples was divided into three parts. The first part was subjected to analyses, while the other two were subjected to the massaging process in a 2000-L volume vacuum massaging machine with a cooling jacket. The massaging machine was filled with the raw material in 60%, while the vacuum amounted to 95%. The machine was programmed into an interval cycle and its effective time of operation was 8 h. Following the division of the massaged samples into two parts, one was subjected to analyses, while the other was put into cylindrical steel cans and pasteurised in a laboratory ultra-thermostat until the temperature of +72°C was

achieved in their geometrical centres. The preserves were subjected to analyses after cooling. In this way, four experimental samples were obtained prepared in three replications: the control, injected with brine, massaged and pasteurised.

Measurement of the pH value. Concentrations of hydrogen ions (pH) with the assistance of the Accumet 15 pH-meter were carried out in each sample. The pH-meter was scaled against three model buffers of pH=4.0; 7.0 and 10.0. In addition, histological specimens were prepared.

Preparation of histological specimens. Cuboids measuring 10x10x30 mm were cut out from the collected muscle samples: the control, injected with the brine, massaged and pasteurised. Next, they were fixed in neutralized formalin to prevent tissue destruction. Fixed muscle samples were embedded in paraffin and cut into 10 µm slices which were placed onto micro slide glass, dried, dewaxed and prepared for staining. A combined slice staining was used applying Delafield haematoxylin and eosin.

Computer image analysis. Investigations of the muscle tissue structure were carried out employing the system of computer image analysis [Dolata *et al.*, 1998; Lu *et al.*, 2000] with the assistance of the MultiScan program. An identical procedure of object identification and analysis was developed for all preparations. The preparation structure was studied at constant microscope magnification (×200) and 10 fields of constant area were analysed from each preparation. The characterization of the images obtained was conducted on the basis of the following parameters of muscle fibres: area, circumference, Feret's diameter H and V, percentage proportion of muscle fibres and their quantity in the analysed field of view. The shape of fibres was determined on the basis of the ratio of H:V diameters. The closer was the value of the quotient to 1, the more regular was the shape of fibres.

Statistical analysis. The numerical data obtained were subjected to statistical analyses using STATISTICA software. The significance of differences was determined by the Fisher test at a significance level of p 0.05.

RESULTS AND DISCUSSION

The pH value of the ham muscles employed in the described investigations ranged from 5.68 to 5.72. According to Tyszkiewicz [1991], the raw material for ham production subjected to thermal treatment should be characterised by the pH value >5.8 but it should not exceed the value of pH=6.2. The concentration of hydrogen ions (pH) of the raw material affects the water holding capacity and production effectiveness (which depends on the former), the binding and consistency of the cooked ham as well as its capacity for the absorption of the curing brine and, hence, the diffusion of salt and curing factors. This type of raw material guarantees the maintenance of a compromise between water holding capacity, capacity to absorb salt and curing agents and stability. In addition, this also exerts influence on the proper colour of the product as well as its shelf-life understood as conditions for the development of bacterial microflora and product sensory quality. Simultaneously, the choice of ham

muscles complying with the above range of pH values allows to eliminate muscles with technological defects of PSE and DFD type [Dolata *et al.*, 2005].

The applied vacuum massaging machine equipped in a cooling jacket enabled employing in this study the curing brine with the temperature of +4°C. It is clear from the review of literature on the subject that the massaging temperature affects ham colour, contrast, cohesion as well as the effectiveness of the finished product [Knipe *et al.*, 1981]. Schopf *et al.* [1995] claim that the optimal temperature, which should be maintained during the plasticizing process of ham muscles, should be about 0°C. In the opinion of the above-mentioned researchers, such temperature ensures the shortening of the duration of the massaging process by about 40%, increases the yield of the finished product, improves its cuttability and colour stability as well as hygienic conditions over the entire production process.

Structure analysis revealed that raw quadriceps muscles of the thigh were characterised by muscle fibres of smaller diameter (Figures 1 and 2) as well as the greater quantity of cells in the examined field of view (Figure 3) in comparison

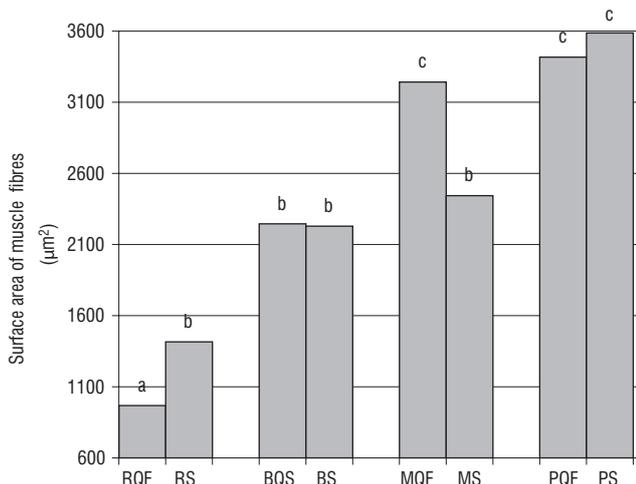


FIGURE 1. Changes in the surface area of muscle fibres caused by: the injection with the curing brine, massaging and pasteurisation (*musculus quadriceps femoris*: raw-RQF, injected with brine-BQF, plasticized-MQF, pasteurised-PQF; *musculus semimembranosus*: raw-RS, injected with brine-BS, plasticized-MS, pasteurised-PS).

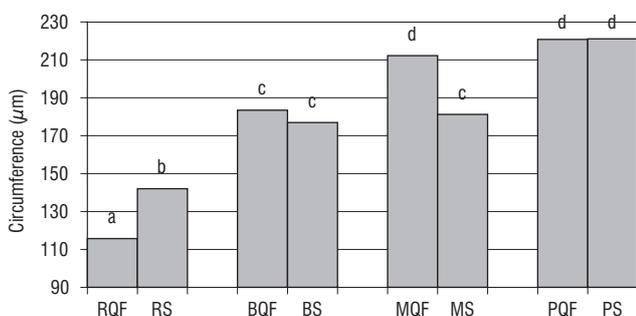


FIGURE 2. Changes in the circumference of muscle fibres caused by: the injection with the curing brine, massaging and pasteurisation (*musculus quadriceps femoris*: raw-RQF, injected with brine-BQF, plasticized-MQF, pasteurized-PQF; *musculus semimembranosus*: raw-RS, injected with brine-BS, plasticized-MS, pasteurised-PS).

with the semimembranous muscles. However, the latter ones exhibited more regular shapes of muscle fibres (Table 1).

TABLE 1. The effect of injection, plasticization and pasteurisation on changes in structure parameters of *musculus quadriceps femoris* (I) and *musculus semimembranosus* (II).

Stage of muscle experiment	Muscle	Feret's diameters (µm)		
		H	V	H/V
Raw	I	41.0 ^a ±1.2	31.9 ^a ±2.7	1.3
	II	45.7 ^{ab} ±2.1	43.0 ^b ±3.9	1.0
Injected with brine	I	61.9 ^d ±7.7	50.5 ^{bc} ±3.4	1.2
	II	52.8 ^c ±4.1	57.4 ^{cd} ±6.4	0.9
Plasticized	I	71.6 ^b ±14.9	60.7 ^{de} ±8.4	1.2
	II	52.6 ^b ±4.5	60.5 ^{de} ±3.8	0.9
Pasteurised	I	64.9 ^{cd} ±4.6	71.4 ^f ±8.0	0.9
	II	70.2 ^{cd} ±4.2	67.0 ^{ef} ±12.8	1.0

* the same letters are used to designate mean values which do not differ significantly at the level of p 0.05

According to Sadowska & Kołodziejka [1994], muscle variability or its absence in the structure of muscles may probably be attributed to the function they play in the organism. It was proved again that changes in the elements of the muscle structure depend on the muscle type. Many researchers, when analysing meat histological structure, reported that muscles from slaughtered animals revealed differences which depended, among others, on the animal species or the type of muscles [Liu *et al.*, 1996; Oryl, 2004; Gajowiecki *et al.*, 2001]. These differences also refer to the muscle texture [Shackelford *et al.*, 1995] and structure [Wiklund *et al.*, 1998] as well as the susceptibility to the massaging process [Motycka & Bechtel, 1983; Shackelford *et al.*, 1989; Gajewska-Szczerbal & Krzywdzińska-Bartkowiak, 2005]. According to Lachowicz *et al.* [2003], each type of muscles requires different massaging parameters.

The performed investigations of the quadriceps muscles of the thigh revealed that the surface of muscle fibres of raw muscles (Photo 1A), muscles injected with the curing brine (Photo 1B), at the termination of the massaging

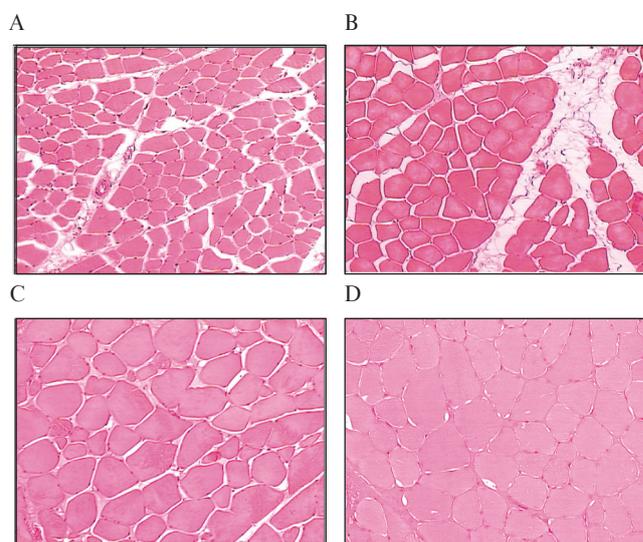


PHOTO 1. Microstructure of the quadriceps muscle of the thigh (*musculus quadriceps femoris*): A – raw, B – injected with brine, C – plasticized, D – pasteurised.

process (Photo 1C) and after pasteurisation (Photo 1D) differed between one another statistically significantly. In addition, a higher dynamics of changes of structural elements was observed in comparison with the semi-membranous muscles. This was confirmed by greater differences in the surface areas obtained during the consecutive stages of the technological process (Figure 1).

The photograph which shows the *semimembranosus* muscle after the injection with the curing brine (Photo 2B) shows the loosening of muscle fibres which resulted in a smaller number of cells in the examined field of view in comparison with the raw muscles (Figure 3). The loosening of muscle fibres can be due to the increase of identical electrical charges following the adsorption of chloride ions which caused longitudinal loosening of myofibrils [Tyszkiewicz, 1991]. Also Dolata *et al.* [2005] reported the loosening of muscle fibres in muscles after their injection.

The massaging process increased the surface area of muscle fibres in the quadriceps muscles of the thigh (Fig-

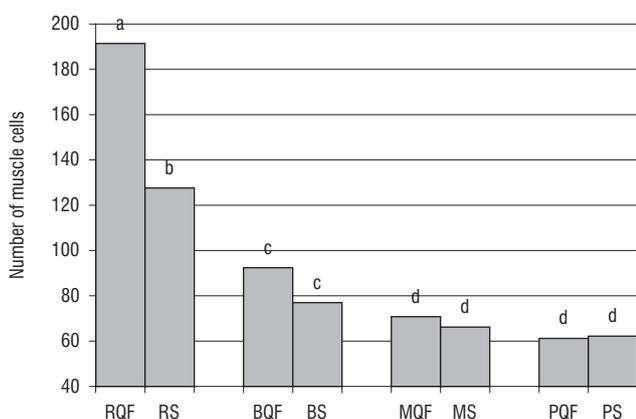


FIGURE 3. Number of muscle cells in the examined field of view of ham muscles: raw, injected with the curing brine, massaged and pasteurised (*musculus quadriceps femoris*: raw-RQF, injected with brine-BQF, plasticized-MQF, pasteurized-PQF; *musculus semimembranosus*: raw-RS, injected with brine-BS, plasticized-MS, pasteurised-PS).

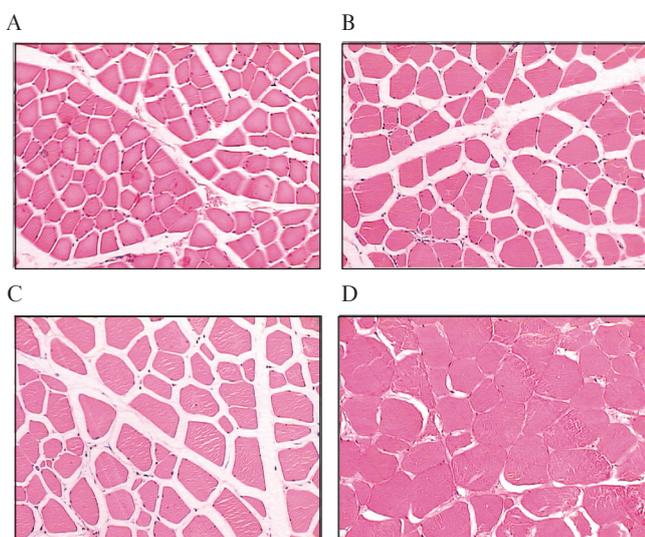


PHOTO 2. Microstructure of the semimembranosus muscle (*musculus semimembranosus*): A – raw, B – injected with brine, C – plasticized, D – pasteurised.

ure 1), circumference (Figure 2) and the percentage proportion of fibres in the examined field of view (Figure 4). This occurred as a result of the partial diffusion of the curing brine into muscle cells (Photo 1C) [Gajewska-Szczerbal & Krzywdzińska-Bartkowiak, 2005; Gajewska-Szczerbal & Krzywdzińska-Bartkowiak, 2006]. These differences were statistically significant. On the other hand, no statistically significant differences were found between the surface area and the circumference of the brine-injected and massaged *semimembranosus* muscle. The *semimembranosus* muscle was characterised by a more regular shape (0.9) in comparison with the *quadriceps* muscle of the thigh (1.2). It was observed that the plasticization process decreased the amount of muscle cells in the examined field of view (Figure 3). Despite this, differences between the two muscles following the injection and massaging were not statistically significant. Statistically significant differences were found between the raw, injected and massaged muscles (Figure 3).

The pasteurisation process did not have any significant

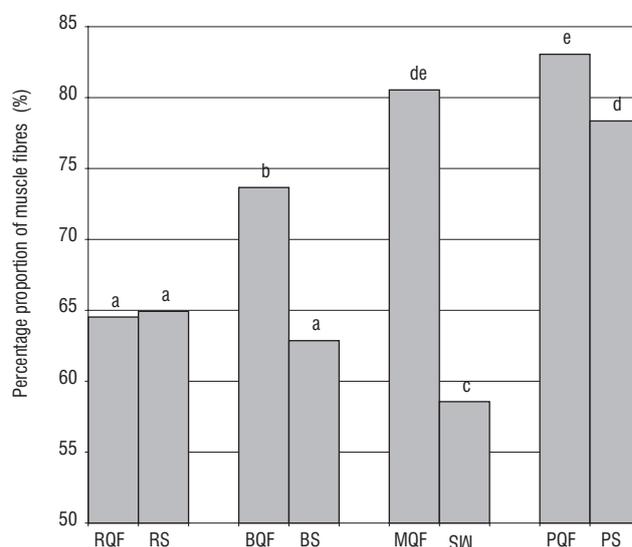


FIGURE 4. Percentage proportion of muscle fibres in the examined field of view of ham muscles: raw, injected with the curing brine, massaged and pasteurised (*musculus quadriceps femoris*: raw-RQF, injected with brine-BQF, plasticized-MQF, pasteurised-PQF; *musculus semimembranosus*: raw-RS, injected with brine-BS, plasticized-MS, pasteurised-PS).

impact on changes in the structure of the quadriceps muscle of the thigh. It can be said that the pasteurisation process resulted in the fixation of the structure which changed in the course of the pasteurisation and massaging. In the case of the *semimembranosus* muscle, the pasteurisation resulted in the increase of the surface area and circumference of muscle fibres, their percentage proportion in the examined field of view at the unchanged number of muscle fibres in comparison with the muscle after the plasticization (Photo 2D). The pasteurisation process led to the levelling out of the cell diameter and decrease of spaces between cells in both of the examined muscles (Photo 1D and 2D).

CONCLUSIONS

On the basis of the investigations performed, it can be concluded that the *quadriceps* muscle of the thigh exhibited a greater dynamics of changes of its structure elements in comparison with the *semimembranosus* muscle following the performed processes of injection and plasticization. The results obtained corroborated the fact that the muscle's histological structure depends, among others, on the type of muscle [Liu *et al.*, 1996; Oryl, 2004; Gajowiecki *et al.*, 2001]. The application of the computer-assisted image analysis allowed to trace and determine the structural changes in the examined *semimembranosus* and *quadriceps femoris* muscles subjected to processes of injection, plasticization and massaging.

The employed injection process of ham muscles resulted in the loosening of muscle fibres and their rounding as well as the increase of the cross section surface area.

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ANALIZA ZMIAN STRUKTURY HISTOLOGICZNEJ MIĘŚNI SZYNKI POD WPLYWEM PEKLOWANIA I OBRÓBKI TERMICZNEJ

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Celem prezentowanej pracy była ocena dynamiki zmian parametrów histologicznych dwóch mięśni świńskich: mięśnia półbłoniastego (*m. semimembranosus*) i mięśnia czterogłowego uda (*m. quadriceps femoris*), pod wpływem procesów peklowania, masowania oraz pasteryzacji, przy zastosowaniu komputerowej analizy obrazu. Analizę struktury mięśni przeprowadzono w oparciu o: powierzchnię i obwód włókien mięśniowych, średnicę Fereta H i V, procentowy udział włókien mięśniowych oraz ich ilość w analizowanym polu widzenia. Ze stosunku H/V określono kształt włókien. Stwierdzono, że mięsień czterogłowy uda wykazywał większą dynamikę zmian elementów struktury w porównaniu do mięśnia półbłoniastego pod wpływem procesów nastrzykiwania i plastyfikacji (tab. 1, rys. 1, 2, fot. 1, 2). W efekcie nastrzykiwania w mięśniach szynek nastąpiło rozluźnienie włókien mięśniowych i zaokrąglenie ich konturów, jak również wzrost pola powierzchni na przekroju. Proces pasteryzacji nie wpłynął na zmiany struktury mięśnia czterogłowego uda, natomiast w mięśniu półbłoniastym pasteryzacja spowodowała powiększenie pola powierzchni i obwodu włókien mięśniowych oraz wzrost ich procentowego udziału w badanym polu widzenia, w porównaniu do mięśnia masowanego (rys. 3, 4, fot. 2). W wyniku pasteryzacji nastąpiło wyrównanie średnicy komórek i zmniejszenie przestrzeni międzykomórkowych w obu badanych mięśniach.