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APPLICATION OF THE NMR TECHNIQUE FOR THE ANALYSIS OF THE WATER STATE IN PICKLED AND PLASTICIZED MUSCLES OF LARGE SLAUGHTER ANIMALS

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Abstract. The paper presents an analysis of the physical and chemical parameters determining the state of water in *musculus longissimus dorsi* and *musculus semimembranosus* pork and beef muscles in the course of the pickling process and the plasticization and pasteurization processes. Values of the relaxation times for the spin-lattice T_1 and spin-spin T_2, pH values, water activity, as well as the content of free water and total water content were determined. Depending on the stage of the experiment, measurements of the spin-spin relaxation times revealed the presence in the examined muscles of free and bound water fractions of various levels of mobility. It was found that the massaging process changes the proportion of the free water and bound water obtained by NMR method. But its molecular dynamics was reduced considerably. In comparison with pickling, the pasteurization process did not change the value of water activity. However, the total water content in muscles decreased, while the content of free water was found increased considerably.

Key words: low field NMR, muscles, water state

INTRODUCTION

Smoked and scalded products manufactured from whole muscles, most frequently pork, are very popular with many customers. On the other hand, there are few smoked and scalded meat products from beef muscles. The above-mentioned meat articles are often manufactured from the *musculus longissimus dorsi* and *musculus semimembranosus* muscles. The quality of final products depends – to a considerable extent – on the processes the muscles are subjected to in order to obtain a good final product. The optimization of these processes is possible thanks to the understanding of changes that take place in muscles during their course.

Changes occurring in the raw material, in particular alterations in the content and state of water binding during the process of injection, massaging and thermal treatment, can be observed by determining such parameters as the pH value, water activity, the content of free and total water. Methods based on the nuclear magnetic resonance (*NMR*) found their application in investigations of food articles because of their non-invasive nature of measurements. They allow studying the physical and chemical properties and monitoring the dynamics of various processes taking place in food [1,7,10]. Using *NMR* techniques, it is possible to determine the content of water in products as well as its influence on the remaining components such as proteins, polysaccharides or fats [9]. One of the NMR methods applied in investigations of muscle tissues is the relaxation in low magnetic fields [1,2].

The recognition of all these characteristics and their proper correction makes it possible to manufacture products from whole muscles which are characterized by high quality and which do not require high expenditure. In addition, it allows selecting appropriate parameters in the course of production of finished products.

MATERIAL AND METHODS

The experimental material comprised pork and beef muscles which were situated in different parts of the carcass. Experiments were carried out on: *musculus longissimus dorsi*, and *musculus semimembranosus.*

The pork muscles were derived from pigs with the after-slaughter weight of about 85 kg, after chilling, without leaf fat and after evisceration, whereas beef muscles were collected from carcasses of 5-year old cows. Samples were collected 48 hours after slaughter, directly in the meat processing plant. The mean temperature of meat samples was 3.5^{o}C and the pH values of the samples were as follows: pork meat – *musculus longissimus dorsi* – 5.65 units and *musculus semimembranosus* – 5.80 units; beef meat – *musculus longissimus dorsi* – 5.57 units and *musculus semimembranosus* – 5.74 units. The brine injected into meat muscles was characterized by the following mean parameters: temperature – 1.3^{o}C, pH value – 7.31, NaCl concentration – 7.81%, and phosphate concentration 0.4%.

After cutting them out, the muscles were divided into four parts. One part was treated as control, while the remaining three parts were injected with brine using a double headed multi-needle injector and the pressure of 0.3 MPa. The injection was carried out three times, obtaining a weight increase of 30%. After the injection, one part of the sample was used for investigations, while the remaining two parts were subjected to the process of plasticization in a vacuum massaging machine (95% vacuum) with the following cycle: 20 minutes massaging and 10 minutes break. The effective massaging time was 6 hours and 20 minutes. After plasticization, one sample was subjected to analyses, while the other was put into

cylindrical cans of 330 g volume, which were subjected to pasteurization at the temperature of 72°C (in geometric centre of sample) for 20 minutes. After pasteurization, the cans were cooled down in tap water to the temperature of 16°C and their content was subjected to analyses after 24 hours.

In this way, four basic samples were obtained: control (S), injected with brine (N), massaged (M) and pasteurized (P). For all four basic samples all physical and chemical investigations were carried out in three replications. Muscle samples for the chemical and physical analyses were collected at each stage of the experiment.

Measurements of spin-lattice T_1 and spin-spin T_2 relaxation times were determined using a pulse *NMR* spectrometer operating at 30 MHz, belonging to the Department of Physics of the Agricultural University in Poznań. T_1 measurements were taken using inversion recovery pulse sequence (π–τ–$\pi/2$) [4]. The distances between RF pulses were changed from 1 to 1000 ms, while the repetition time was 6 s. Five signal accumulations were applied and the values of the relaxation times were calculated employing the following formula:

$$\boldsymbol{M_z} = \boldsymbol{M}_0\left(1 - 2\exp\left(-\boldsymbol{\tau}/\boldsymbol{T}_1\right)\right) \tag{1}$$

where: M_0 and M_z are, respectively, equilibrium and current magnetization values.

The T_2 relaxation times were measured using a *CPMG* pulses train [3, 6]. Sequences of 50 spin echoes were applied with distances between impulses (*TE*) of 2 ms and a repetition time of 6 s. Ten signal accumulations were applied and the values of the relaxation times were calculated employing the following dependence:

$$\boldsymbol{M}_{x,y} = \sum_{i-1}^{n} \boldsymbol{p_i} \exp\left(-\boldsymbol{TE}/\boldsymbol{T}_{2i}\right) \tag{2}$$

where: M_0 is equilibrium and current magnetization values and p_i – the fraction of water protons relaxing with the T_{2i} time.

Measurements were carried out at the temperature of +20°C.

The concentration of hydrogen ions was determined with the aid of the Accumet 15 pH meter equipped in a combined electrode.

Water activity was measured with the assistance of the AQUASPECTOR-1 measuring device. The principle behind the measuring method involves the determination of the value of a parameter after the establishment of the humidity equilibrium in the measuring chamber over the examined sample [8]. It is expressed by the ratio of the water vapour pressure over the product (p) to the vapour pressure over clean water (p_o).

$$a_W = \frac{p}{p_0} \tag{3}$$

The modified Grau-Hamm impression method was applied to determine free water [5]. The modification consisted in the increase of the sample weight and was enforced by technical considerations. Muscle samples in the shape of cylinders weighing about 1.5 g were cut out by a special tube knife of 2.54 cm in diameter and then placed for 20 minutes under the load of 1 kg on the Whatman 4 absorbent paper. The orientation of the muscle fibers was parallel to the force direction. Next, the drip area on the absorbent paper was measured using a planimetre, and the amount of drip was calculated using the following formula:

$$W_W = \frac{0.545 \cdot S}{m} \tag{4}$$

where: W_w – free water (%), S – stain surface area (mm), m – sample weight (g), 0.545 – correction factor.

The total water content in muscles was determined with the oven-dry method at the temperature of 105 ^{o}C. The results were calculated as the difference between the 100% sample weight and the dry matter content.

$$W = 100 - S_m \tag{5}$$

where: W – water content (%), S_m – dry matter content (%).

RESULTS AND DISCUSSION

The results of chemical and physical analyses carried out in the course of the individual stages of the experiments are shown in appropriate figures.

In order to determine the quantitative and qualitative changes of water in the examined muscles, alterations in the values of the relaxation times obtained for the samples at all phases of the process of pickling and pasteurization were analyzed.

Value changes in the spin-lattice T_1 relaxation time reflecting the quantity of the free water in muscles in relation to the amount of bound water are presented in Figure 1.

The untreated *longissimus dorsi* muscles showed longer relaxation time than the *semimembranosus* muscles. The longest spin-lattice time was found in the pork *longissimus dorsi* muscle. The injection process resulted in an increase of the T_1 value of all muscle types. A further increase in the T_1 value was found in pork muscles after massaging. In the case of beef muscles, this parameter increased only in the *longissimus dorsi* muscle, whereas in the case of the beef *semimembranosus* muscles,

T_1 values decreased reaching the values observed in the untreated muscle. The pasteurization process resulted in a shortening of the relaxation times in both pork muscles and in the beef *semimembranosus* muscle.

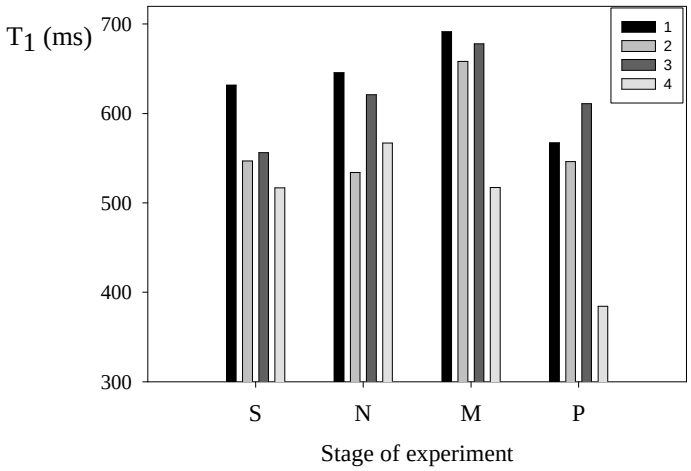

Fig. 1. Changes in the T_1 value for individual phases of the experiment (S - untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 – beef musculus semimembranosus)

The examined muscles were characterized by two spin-spin relaxation times. The short component of this time reflected the mobility of bound water molecules, whereas the long one derived from free water protons. Changes in these relaxation times are presented in Figures 2 and 3.

In the case of the pork untreated muscles, bound water showed greater mobility than in beef muscles. The injection and plasticization processes caused an increase in the value of the short component of the T_{21} spin-spin relaxation times. Following the pasteurization process, in comparison with the untreated muscles, the mobility of bound water protons increased. It was found that, in comparison with the *longissimus dorsi* muscles, the molecular dynamics of bound water in the *semimembranosus* muscles was considerably limited. The greatest changes in all the stages of the performed experiment were observed in the beef *semimembranosus* muscles.

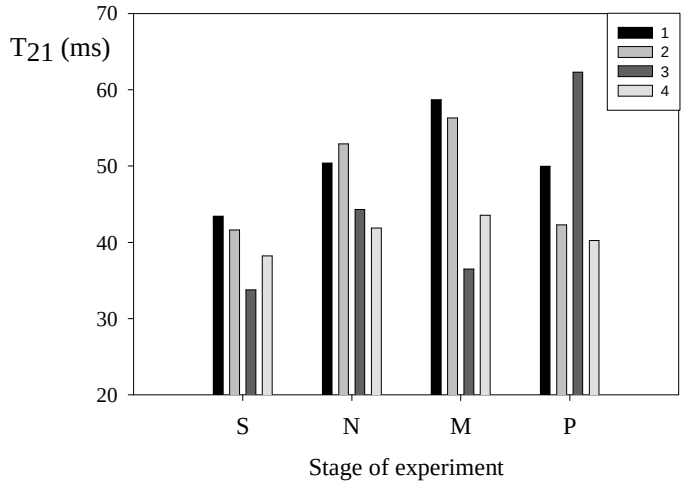

Fig. 2. Changes in the value of the short component T_{21} value for individual phases of the experiment (S - untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 – beef musculus semimembranosus)

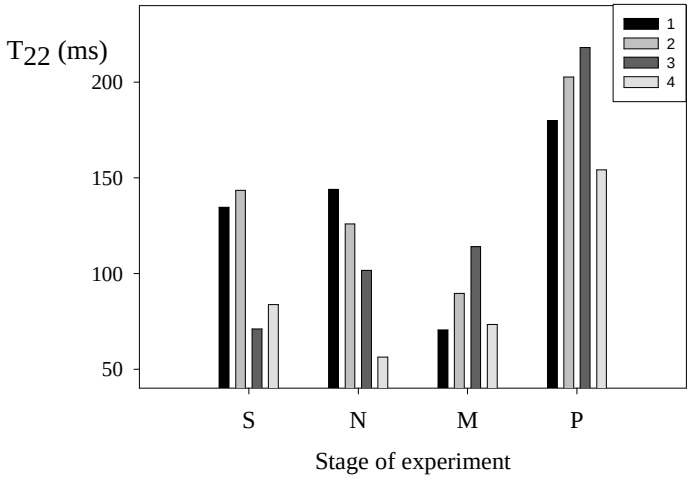

Fig. 3. Changes in the value of the long component T_{22} value for individual phases of the experiment: (S – untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 - beef musculus semimembranosus)

Analysis of the values of the long component of the spin-spin relaxation times showed that pork muscles contained water characterized by much greater dynamics than beef muscles. The mobility of water protons in the untreated *longissimus dorsi* muscles was smaller than in the *semimembranosus* muscles. The mobility of water molecules declined in the pork *longissimus dorsi* and *semimembranosus* muscles after their injection with the pickling brine. Massaging was found to reduce significantly the molecular dynamics of free water because it became bound as indicated by the lengthening of the T_{21} spin-spin relaxation time (Fig. 2). Following the pasteurization process connected with muscle thermal treatment, the T_{22} values were found increased.

Investigations conducted using the *NMR* technique allowed analyzing the state of water in muscles at the molecular level. After completing all phases of the trial, it can be stated that the beef *longissimus dorsi* muscles were characterized by the highest content of free water in relation to the amount of bound water. Both free water and bound water in this muscle exhibited the highest mobility. The beef *semimembranosus* muscles showed the lowest amount of free water in relation to the amount of bound water. At the same time, the molecular dynamics of both water fractions in this muscle was inhibited the most.

The obtained results were compared with the changes of parameters determining the state of water in muscles measured using classical methods. Value changes in the concentration of hydrogen ions in muscles in the consecutive phases of the experiment are shown in Figure 4.

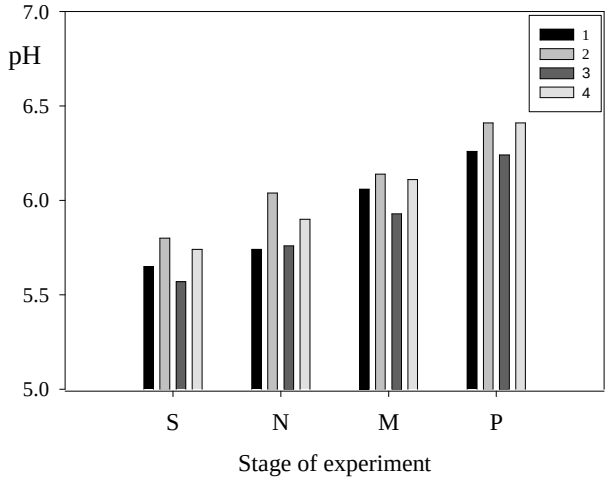

Fig. 4. Changes in pH values in consecutive phases of the experiment: (S - untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 – beef musculus semimembranosus)

Raw muscles differed with regard to their pH values, with higher values determined in pork than in beef muscles. Higher values were determined in both pork and beef *semimembranosus* muscles, on average 5.80 and 5.74, respectively, in comparison with mean pH values of 5.65 and 5.50 found in the *longissimus dorsi* muscles.

A difference was found in the pH value in individual stages of the experiment for each muscle. When examining the observed changes in the pH values in muscles, they were found increased as a result of the applied injection, plasticization and pasteurization processes. The highest difference was noted between the beef untreated and pasteurized muscles; the difference reached 0.67 pH units. In addition, the type of muscle was also found to affect the pH value.

Changes in the water activity in the examined muscles are presented in Figure 5.

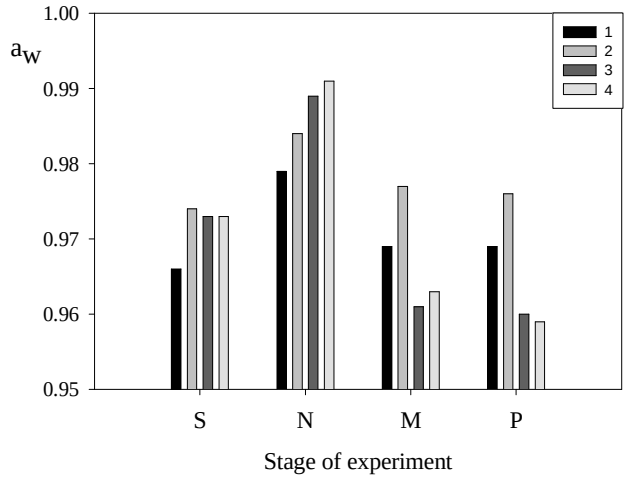

Fig. 5. Changes in values of water activity in consecutive phases of the experiment: (S – untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 – beef musculus semimembranosus)

The lowest value of water activity among the raw muscles (a_w = 0.966) was found in the pork *longissimus dorsi* muscle. As a result of the injection process, all muscles were characterized by the highest water activity of all the assessed experiment phases. However, the plasticization treatment decreased the value of this parameter considerably. After the brine injection, both beef muscles were characterized by a higher a_w value than the pork muscles. On average, it amounted to 0.989 in the *longissimus dorsi* muscle and 0.991 in the *semimembranosus* muscle. The plasticization process decreased significantly the water activity in the examined muscles, especially in the beef muscles. The recorded decrease was the lowest

after the massaging process in the beef muscles, on average by 0.028 in comparison with the raw muscles.

The injection process was found to increase significantly the water activity in all types of the examined muscles. Differences in the water activity were found between muscles of both of the examined animal species. In the case of the beef muscles, differences between the a_w values in the two examined muscle types were not significant.

The amounts of free water in the examined muscles in each of the experimental phases are presented in Figure 6.

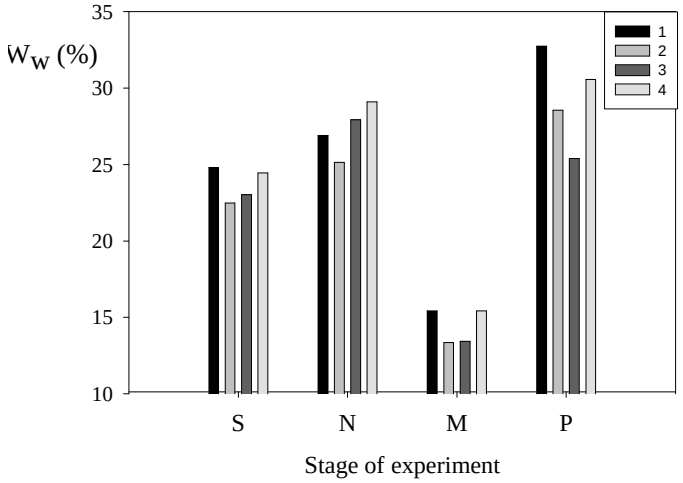

Fig. 6. Changes in the content of free water in consecutive phases of the experiment: (S – untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 – beef musculus semimembranosus)

The obtained results revealed varying contents of free water in the muscles in each of the experimental phases. Untreated muscles contained, on average, from 22.49% of free water in the pork *semimembranosus* muscle to 24.81% – in the pork *longissimus dorsi* muscle. Its quantity in beef muscles was similar and differences between the muscles were not significant statistically. The untreated pork *longissimus dorsi* muscle was characterized by a higher content of free water than the beef one; their respective quantities were: 24.81% and 23.03%.

The injection with the pickling brine increased the content of free water in all the examined muscles. Its quantity decreased by nearly half after massaging. The highest amounts of free water were found in pasteurized muscles. After injection, the content of free water increased from 24.45% to 29.09% in the beef *semimembranosus*

muscle, whereas after massaging, it decreased by 13.66%. The pasteurization process increased the amount of free water in this muscle by 15.13% in relation to the massaged muscle. The distribution of free water in each phase of the experiment for the remaining muscles was similar.

Figure 7 presents changes in the total water content in the muscles. The untreated muscles differed with regard to the water content but those differences were not significant. The highest water content was found in the raw pork *semimembranosus* muscle. *Semimembranosus* muscles of both animal species contained more water than the *longissimus dorsi* muscles, and beef muscles contained less water than pork muscles. The process of brine injection resulted in an increase of the water content in all examined muscles. This increase was the highest in the pork *longissimus dorsi* muscle and the difference, in comparison with the raw muscle, was 3.1%. A similar phenomenon occurred in the remaining muscles. The pasteurization process reduced significantly the content of water in all the muscles, which was caused by thermal drip. The lowest total water content was found in the pork *semimembranosus* muscle, which was probably caused by the highest thermal drip of all the muscles. The process of pasteurization caused the largest differences in the *semimembranosus* muscles, while the *longissimus dorsi* muscles were found to be the most differentiated after the massaging process. It was also found that the observed changes in the total water content were most affected not by the type of muscles but by the successive phases of the experiment.

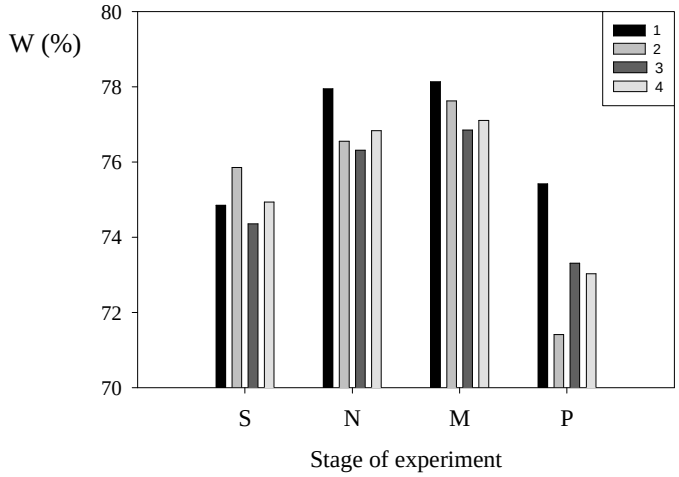

Fig. 7. Changes in the total water content in consecutive phases of the experiment: (S – untreated muscle, N – muscle injected with brine, M – massaged muscle, P – pasteurized muscle), (1 – pork musculus longissimus dorsi, 2 – pork musculus semimembranosus, 3 – beef musculus longissimus dorsi and 4 – beef musculus semimembranosus)

The *NMR* technique applied in the experiment allowed detailed monitoring of the state of water in consecutive stages of the experiment. The performed measurements of relaxation times made it possible to follow closely quantitative and qualitative changes in muscles. It can be said that the injection with the pickling brine did not change mutual relationships between the amount of free and bound water in pork muscles as implied by the good absorption of the solution. In the case of beef muscles, free water was found increased in comparison with bound water at this stage of the process. The massaging process decreased the content of free water in muscles (Fig. 6) determined using the impression method. In addition, this method, generally speaking, failed to distinguish the type of muscles and the species of animals. However, investigations carried out on the molecular level (*NMR*) showed that the massaging process removed water from intra-tissue spaces and a distinct increase in the values of the spin-lattice relaxation times in both pork muscles and in the beef *longissimus dorsi* muscle was observed. Moreover, the beef muscles – in comparison with pork muscles – were characterized by decreased molecular dynamics of bound water.

CONCLUSIONS

1. The technique based on the *NMR* phenomenon allows quantitative assessment of free and bound water in the muscle and of the molecular dynamics of both water fractions in the process of curing and pasteurization.

2. The processes of brine injection, plasticization and pasteurization increased both pH values and free water content in all the examined muscles, in particular, in the pork muscles.

3. The processes of plasticization and pasteurization reduced significantly the water activity in all the examined beef muscles.

4. The content of free water was characterized by the highest dynamics of changes as a result of the applied processes of brine injection, plasticization and pasteurization. Brine injection increased this dynamics, plasticization decreesed it and pasteurization again increased it significantly.

REFERENCES

1. **Bertram H.C., Andersen H.J., Karlsson A.H.:** Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding of pork. Meat Sci., 57, 125-132, 2001.
2. **Bertram H.C., Rasmussen M., Busk H., Oksbjerg N., Karlsson A.H., Andersen H.J.:** Changes in Porcine Muscle Water Characteristics during Growth – An ^{1}HLow-Field NMR Relaxation Study. J. Magnet. Reson., 157, 267-276, 2002.

3. **Carr H.Y., Purcell E.M.:** Effects of diffusion on free precession in nuclear magnetic resonance experiments. Phys. Rev., 94, 630-638, 1954.
4. **Fukushima E., Roeder S.B.W.:** Experimental Pulse NMR. A Nuts and Bolts Approach. Addison Wesley Publishing Company, London, 1981.
5. **Honikel K.O.:** Reference methods for the Assessment of Physical Characteristics of Meat. Meat Sci., 49, 447-457, 1998.
6. **Meiboom S., Gill D.:** Modified spin-echo method for measuring nuclear relaxation times. Rev. Sci. Instrum., 29, 688-691, 1958.
7. **Pedersen H.T., Berg H., Lundby F., Engelsen S.B.:** The multivariable advantage in the determination in meat by bench-top NMR. Innovative Food Sci. & Energing Technol., 2, 87-94, 2001.
8. **Pijanowski E., Dłużewski M., Dłużewska A.:** General Food Technology (in Polish) WNT, Warszawa, 1990.
9. **Sorland G.H., Larsen P.M., Lundby F., Rudi A.-P., Guiheneul T.:** Determination of total fat and moisture content in meat using low field NMR. Meat Sci., 66, 543-550, 2004.
10. **Tornberg E., Wahlgren M., Brondum J., Engelsen S.B.:** Pre-rigor conditions in beef under varing temperature and pH-falls studied with rigometer, NMR and NIR. Food Chem., 69, 407-418, 2000.

WYKORZYSTANIE TECHNIKI MRJ DO ANALIZY STANU WODY W PEKLOWANYCH I PLASTYFIKOWANYCH MIĘŚNIACH DUŻYCH ZWIERZĄT RZEŹNYCH

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Streszczenie. W pracy przedstawiono analizę fizycznych i chemicznych parametrów określających stan wody w mięśniach świńskich i bydlęcych *musculus longissimus dorsi*, *musculus semimembranosus* podczas procesu peklowania, plastyfikacji i pasteryzacji. Określano wartości czasów relaksacji spin-sieć T_1 i spin-spin T_2 oraz wartości pH, aktywności wody i zawartość wody wolnej i ogólną zawartość wody. Pomiary czasów relaksacji spin-spin ujawniły obecność frakcji wody wolnej i wody związanej w mięśniach o zróżnicowanej mobilności w zależności od etapu doświadczenia. Stwierdzono, że proces masowania zmienia proporcje wody wolnej i wody związanej określane metodą MRJ a dynamika molekularna wody jest znacznie obniżona. W porównaniu do peklowania, proces pasteryzacji nie zmienia wartości aktywności wody. Stwierdzono jednak obniżenie ogólnej zawartości wody w mięśniach i znaczne zwiększenie zawartości wody wolnej po tym procesie.

Słowa kluczowe: MRJ, mięśnie, stan wody