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ANALYSIS OF WATER PROPERTIES IN SELECTED PORCINE MUSCLES SUBJECTED TO THE PROCESS OF INJECTION CURING

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Abstract. The presented study discusses results of investigations carried out on two porcine muscles: the triceps muscle of the arm (*musculus triceps brachii*) and the semimembranosus muscle (*musculus semimembranosus*). Raw muscles, muscles after brine injection under different pressures and after plasticisation were assessed. On the basis of the results obtained, variations in the water content as well as differences in the pH value and the quotient of the sodium chloride and water (S/W) concentrations in both types of muscles and at each phase of the experiment were observed. The use of different injection techniques with the curing brine (low pressure and spray injection) affected the free drip from the muscles after the injection as well as the value of the P/W (protein : water) quotient The increase of water activity in the *m. triceps brachii* was observed only after the plasticization process, whereas in the *m. semimembranosus* – already after the injection with the curing brine. The utilization of the nuclear magnetic resonance (NMR) phenomenon allowed to find differences in the organisation of water in both of the muscles examined in successive process phases of the experiment, although they were not observed to occur between the raw muscles.

Keywords: muscles, curing techniques, water properties, NMR

INTRODUCTION

The essence of the meat curing process consists in the diffusion of the water solution of curing compounds deep into muscle cells. The phenomenon can be attributed to the difference in the osmotic pressures between the cell content and the brine found in the intercellular spaces. The moment of equalisation in the concentration of salts making up the brine terminates the physical-chemical process of curing. Consequently, it can be said that this event is associated with an increase in the end-product yield and with an improvement of the economic effectiveness of the production process [6]. This can be attributed to the applied

technique of brine introduction, which pre-conditions, among others, the water holding capacity of the meat of a specific pH value and chemical composition [4]. At present, brine is introduced into meat with the assistance of special multi-needle injectors. According to Olszewski [7], the pH of the cured porcine meat should range from 5.7 to 5.8. In order to reach these values and to make sure that the curing process proceeds correctly, the meat should be characterized, prior to curing, by the value of hydrogen ion concentration ranging from 5.5 to 5.8 pH units.

A higher pH value is directly associated with a higher water holding capacity and a better capability of meat to bind water. The brine component that exerts a significant influence on meat water holding capacity and, at the same time, prolongs its storage life, is sodium chloride, as it reduces water activity (a_w). Simultaneously, it is possible to notice a distinct correlation between the a_w value and the percentage quotient of NaCl and water (*S/W*) in meat products. Water activity further depends significantly on the total meat water content as well as on the quotient value of the percentage protein and water contents (*B/W*) [4,5].

One of the non-invasive and non-destructive methods of assessment of the free water content in a system is a technique based on the nuclear magnetic resonance (NMR). In the case of muscle tissues, increased water content results in an extended spin–lattice relaxation time T_1. The same effect is observed in the case of weakly bound water on the surface of muscular proteins. Depending on the quantity of water arrested in the system, values of the spin–lattice relaxation time measured using the NMR technique range from 500 to 700 ms at the applied frequencies of approximately 10-40 MHz [2,8,9].

The objective of this research project was to analyse water properties in two selected porcine muscles, of quite different topographic locations in the carcass, which were subjected to curing using the injection method. Bearing in mind the fact that both pressures as well as sizes of brine droplets flowing out of needles of the injection head exert a significant impact on the meat water holding capability [2], the experiments performed employed two different injection devices of varying design.

An attempt was made to compare physical-chemical parameters characterizing the cured meat with the values of the spin–lattice relaxation time T_1 which affect water properties on the molecular level.

MATERIAL AND METHODS

The experimental material comprised two porcine muscles: the triceps muscle of the arm (*musculus triceps brachii*) situated on the outer side of the front limb and the semimembranosus muscle (*musculus semimembranosus*) forming a part of the ham muscles. Forty-eight hours after slaughter, muscles chilled to the

temperature of +4°C were subjected to injection curing employing two different types of multiple-needle injection devices: a high-pressure (spray) device and a low-pressure one.

The amount of brine used to inject the muscle in the experiment constituted 50% of the muscle weight and its temperature was +4°C, while the pH value – 6.95. Both injection devices applied brine of identical chemical composition containing: 3.10% protein, 7.32% sodium chloride and 0.84% polyphosphates calculated per P_2O_5.

The first of the devices introduced the curing brine into the muscle volumetrically under the pressure of 1.2 MPa through a set of needles of which each had 14 holes of 0.6 mm diameter situated at various heights. The applied high pressure and small aperture diameters resulted in the brine being sprayed in the form of micro-droplets simultaneously in the entire muscle. The injection was conducted once and started when needles reached the deepest portions of the muscle.

The second injector used in the experiment was a low-pressure device equipped with a continuous action pump which pumped the brine into the muscle under the pressure of 0.4 MPa through needles with 4 holes of 1 mm diameter each. The brine flow from needles continued as long as the needles remained in the muscle. In order to introduce the intended quantity of brine of 50% in relation to the initial muscle weight, the muscles were injected with brine 9 times. After the injection, muscles were subjected to plasticization (massaging) in a vacuum massaging-device. The plasticization process took 8 hours and was carried out in a 95% vacuum. The effective massaging time amounted to 4 hours and was conducted in 15-minute work-cycles followed by 15 minutes of break. The muscle temperature during massaging was +4°C and at massaging termination +5°C ÷ +6°C.

In order to determine the amount of the brine free drip from injected muscles, they were weighed directly after the injection and also 1.5 hours after it. The value of the free drip was given in percentages. Next, the pH value in muscle samples against three standards was determined using the Accumet -15 pH-meter. Water activity in the muscles was measured with the assistance of the Aquaspector–1 apparatus whose principle is based on the measurement of vapour pressure after achieving relative humidity equilibrium in the test chamber above the sample. The water content in muscle samples was assessed during each experimental phase using the drier method. Samples were subjected to the action of hot air at the temperature of 105°C until they reached constant weight. In addition, the total protein content (N x 6.25) was estimated by the Kjeldahl method and sodium chloride – by the Mohr method using silver nitrate [3]. Last but not least, quotients of protein and water contents (P/W coefficient) as well as sodium chloride and water contents (S/W coefficient) were calculated. The results obtained were subjected to the two-way analysis of variance. The significance of

differences between means was determined for the level of $p \leq 0.05$ on the basis of the least significant difference (LSD).

Muscle samples to be used for measurement of the relaxation time T_1 with the assistance of the NMR technique were collected at each stage of the experiment by cutting cylinders 15 mm in diameter and 10 mm high out of the muscles. The cylinders were placed at once in measurement test tubes in such a way that muscle fibres ran perpendicular to the direction of the constant magnetic field B_o. The samples were then sealed and stored at the temperature of $+4^oC$. NMR investigations were performed 24 hours after sampling.

Measurements of the spin–lattice relaxation time T_1 were carried out using an NMR pulse spectrometer operating at a frequency of 30 MHz. Measurements were taken using the inversion recovery method (π-τ-$\pi/2$); the inversion time was changed from 1 to 1500 ms, the repetition time was 6 s. Five signal accumulations were performed for each sample and 32 free induction delays (FID) were collected each time. The total of 70 points were selected for the analysis at each FID signal and measurements were taken at room temperature. T_1 values were calculated using the CracSpin software employing the spin grouping method [11].

It was found that, for all the samples examined, the magnetisation recovery was single-exponential, hence all the systems were characterized by one spin-lattice relaxation time T_1.

RESULTS AND DISCUSSION

The degree of binding of the curing brine by tissue structure was assessed by the size of the free drip of the unbound brine 1.5 hours after the injection of the muscles examined.

Table 1 presents the values of this parameter for both of the muscles examined

Table 1. The size of the free drip from the examined muscles cured using two types of injectors (%)

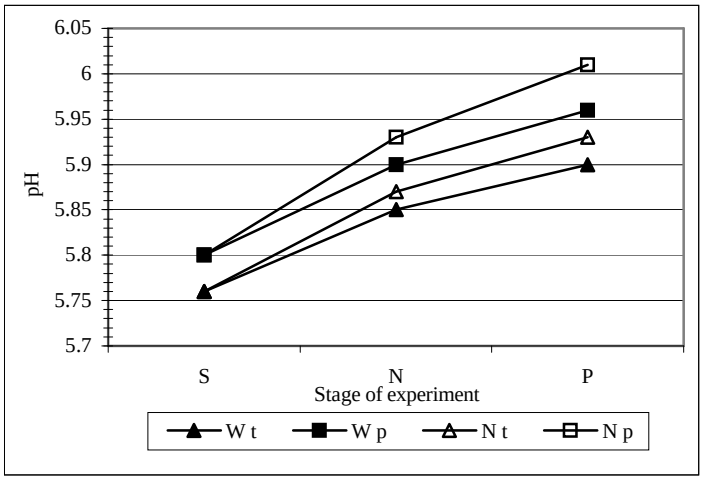
Index	Stage of muscle evaluation	Injector type			
		high pressure injector	low pressure injector	high pressure injector	low pressure injector
		triceps muscle		semimembranosus muscle	
free drip	injected muscle (N)	2.97[a]	8.24[b]	2.17[a]	6.90[b]

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

The experiment allowed to conclude that the percentage amount of the free drip from muscles into which brine was introduced under high pressure (1.2 MPa) using the spay injector was 2.5-3.5 times smaller in comparison with muscles into which brine was injected using the pressure at 0.4 MPa. This finding corroborates observations of other researchers who also reported that muscle tissue absorbs brine more effectively when it is injected into the muscle under high pressure, provided that it is also sprayed in the muscle in the form of micro-droplets by apertures with a fraction of a millimeter in diameter [1].

When comparing the two muscles, i.e. the triceps muscle of the arm and the semimembranosus muscle, it was found that, irrespective of the pressure applied, the observed free drip was higher in the former than in the latter of the muscles examined. If the amount of the free drip from the *m. triceps brachii* is assumed as 100%, approximately 23% less curing brine was found in the *m. semimembranosus* introduced there under high pressure and about 18% less when it was injected using the low pressure device. This could probably be attributed to the lower content in the semimembranosus muscle of proteins swelling as a result of adsorption of chloride ions from the curing brine [10].

The study showed that the pH value of raw muscles (S) before brine injection was 5.76 for the *m. triceps brachii* and 5.80 for the *m. semimembranosus,* and the difference between these values was statistically non-significant (Fig. 1).



Legend: S – raw muscles; N – muscles after injection with brine; P – plastified muscles; W – high pressure injector; N – low pressure injector; t – triceps muscle of arm; p – semimembranosus muscle.

Fig. 1. Changes in the concentration of hydrogen ions in the muscles examined

It can, therefore, be said that the concentration of hydrogen ions in the tested muscles was optimal for the proper course of the curing process [7]. The introduction of the brine (N) increased the pH value of the triceps muscle of the arm from 5.76 to 5.85 and 5.87 units and of the semimembranosus muscle – from 5.80 to 5.90 and 5.93 pH units, respectively, using the high pressure and low pressure injectors. The difference between the muscles before and after brine injection was statistically significant. Both muscles after brine injection using the pressure of 1.2 MPa were characterized by lower pH values than those into which the brine was introduced using the pressure of 0.4 MPa. The process further increased the pH values of the examined muscles by an average of 0.05 and 0.06 in the *m. triceps brachii* and by 0.06 and 0.08 - in the second of the tested muscles using the high- and low-pressure brine injection, respectively. It was found that the semimembranosus muscle, at each stage of the experiment, was characterized by higher pH values and differences between muscles after injection and massaging were statistically significant. The analysis of variance revealed that differences in the pH values of raw and brine injected muscles, raw and massaged muscles, as well as injected and massaged muscles, were statistically significant. On the other hand, the injection techniques applied failed to show any statistically significant impact.

Table 2 shows values of parameters illustrating the state of water in the examined muscles in the individual phases of the experiment. In order to supplement data characterizing the cured muscles, the total protein (N x 6.25) and sodium chloride contents re also included.

The smallest quantities of water were found in the raw muscles. The triceps muscle of the arm contained 75.09% water, i.e. 1.25% more than the *semimembranosus* muscle and the difference between them was statistically significant. It was found that the first of these muscles contained more water after the high-pressure injection than after the low-pressure one, but the differences were not statistically significant. The *m. semimembranosus* was characterised by a lower water content at all the phases of the experiment. It contained more water when the curing brine was introduced under low pressure than after high-pressure injection. Also after plasticization, the difference in the total water content was statistically significant depending on the applied technique of the curing brine injection.

No statistically significant differences were observed in water activity between the examined raw muscles. After brine injection, the a_w value increased, and differences between raw and injected muscles were statistically significant. The increase of water activity was higher when the low- and not the high-pressure injector was applied, irrespective of the type of muscles. Plasticization was found to exert a significant influence on the further increase of the a_w value in the triceps muscle of the arm. Statistically significant differences were recorded between the

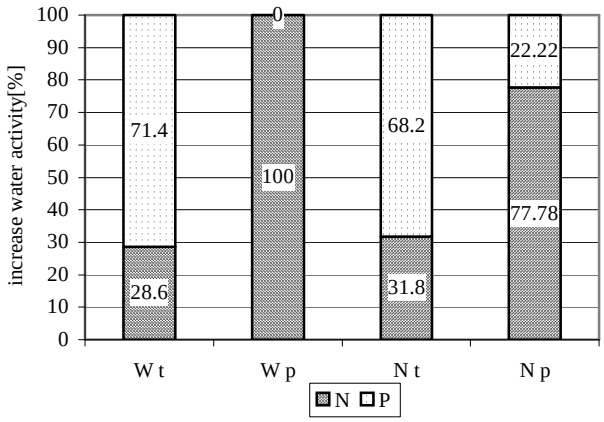
injected and the plasticized muscles. In addition, the technique of the curing brine introduction into the triceps muscle of the arm was found to have a statistically significant effect on the a_w value.

Table 2. Value changes of the physical-chemical parameters determined in raw (S), injected (N) and plasticized (P) muscles in relation to the brine injection method and type of muscle

Index	Stage of muscle evaluation	Injector type			
		high pressure injector	low pressure injector	high pressure injector	low pressure injector
		triceps muscle of the arm		semimembranosus muscle	
Water (%) *W*	S	75.09[a]		73.84[b]	
	N	77.56[c]	75.00[d]	77.76[c]	76.27[e]
	P	77.39[c]	75.54[d]	77.45[c]	77.18[c]
Water activity a_w	S	0.955[a]		0.953[a]	
	N	0.959[b]	0.969[c]	0.969[c]	0.969[c]
	P	0.960[b]	0.975[d]	0.967[c]	0.971[d]
Protein (%) *B*	S	19.57[a]		20.97[b]	
	N	16.10[c]	15.48[d]	16.39[c]	15.77[d]
	P	16.10[c]	14.73[e]	16.24[c]	14.79[e]
B/W	S	0.262[a]		0.284[b]	
	N	0.207[c]	0.200[c]	0.215[d]	0.209[c]
	P	0.207[c]	0.190[e]	0.213[d]	0.192[e]
NaCl (%) *S*	S	0.77[a]		0.53[b]	
	N	2,07[c]	2,05[c]	2,08[c]	2,09[c]
	P	2.82[d]	2.80[d]	2.38[e]	2.34[e]
S/W	S	0,010[a]		0,007[b]	
	N	0.027[c]	0.027[c]	0.028[c]	0.028[c]
	P	0.036[d]	0.036[d]	0.031[c]	0.030[c]

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

If the increase in the water activity in the final phase of the experiment is assumed as 100% in comparison with the raw muscles and recalculating its changes after brine injection and plasticization, it was found that the increased water activity in the *m. triceps brachii* was caused, primarily, by plasticization, whereas in the second of the examined muscles, i.e. *m. semimembranosus* – by the introduction of brine (Fig. 2).



Legend: N – muscles after injection with brine; P – plastified muscles; W – high pressure injector; N – low pressure injector; t – triceps muscle of arm; p – semimembranosus muscle.

Fig. 2. Relative increase of water activity in consecutive phases of the experiment in comparison with raw muscles (%)

The protein content depended both on the type of the cured muscle and on the technique of brine introduction (Table 2). The highest quantities of crude protein were found in raw muscles. When comparing the two curing techniques, it was observed that both muscles contained more protein when injected with brine using the spray injector than when the other technique was used. The level of protein did not change significantly after massaging. On the other hand, in the case of muscles in which the curing brine was introduced using 0.4 MPa pressure and massaged, the amount of protein after plasticization was lower than before the treatment and the differences were statistically significant. This could have been caused by increased and gradual, in comparison with the first method, extraction of proteins from muscles, which is a prerequisite for the development of an 'adhesive' which ensures the proper binding of slices of the finished meat product.

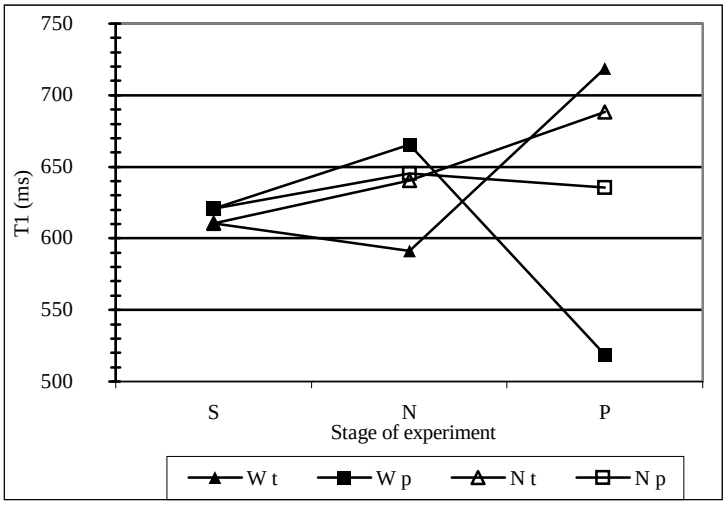
The value of the B/W quotient declined with the increase of water activity [4]. In the experiment presented, the highest *B/W* coefficient was observed in the raw

muscles, characterized by the lowest water activity (Tab. 2). It was found that the difference between quotient values calculated for the two muscles examined was statistically significant. The injection with brine resulted in a statistically significant decrease in the *B/W* value. Muscles into which brine was introduced using the spray method were characterized by a higher *B/W* value in comparison with the low-pressure injection. The value of this parameter was the lowest after plasticization. Differences in values of *B/W* quotients were statistically significant in relation to the curing technique employed.

One of the key constituents of the curing brine, preconditioning good meat water holding capacity and durability of the finished product by reducing, among others, its water activity, is sodium chloride. Raw muscles examined in this experiment contained varying and significantly differing statistically quantities of sodium chloride: the triceps muscle of the arm – the average of 0.77%, whereas the semimembranosus muscle – 0.50% (Tab. 2). The introduction of brine resulted in more than 2.5 times increase of its content in the *m. triceps brachii* and 4 to 4.5 times increase in the *m. semimembranosus*. Both muscles contained more NaCl after plasticization than after injection and this difference was statistically significant. The triceps muscle of the arm contained more sodium chloride and the differences between the examined muscles were statistically significant. It was further found that, in the experiment performed, the concentration of the sodium chloride, as was the case with water activity in the cured muscles, was affected significantly by the type of the examined muscle and not by the pressure used to introduce the brine.

The value quotient of the sodium chloride and water (S/W) content calculated for the *m. triceps brachii* before brine injection was 30% higher in comparison with the *m. semimembranosus* and the difference between the muscles was statistically significant. The introduction of brine exerted a significant influence on the increase of the quotient value in both the muscles. However, in the case of the triceps muscle of the arm, the S/W value increased by 0.017, while in the second of the experimental muscles, this increase amounted to 0.021. The application of plasticization resulted in a further, statistically significant, increase in the *S/W* coefficient only in the triceps muscle of the arm. The value of the discussed coefficient after massaging was affected only by the type of the cured muscle.

The research results discussed above indicate that the curing and plasticization processes applied exerted a varying influence on the values of parameters associated with water properties in both of the muscles examined. That is why an attempt was made to analyse changes in the spin-lattice relaxation times T_1 in these systems. Value changes of relaxation times T_1 for raw muscles as well as those treated with the curing brine and subjected to plasticization are shown in Figure 3.



Legend: N – muscles after injection with brine; P – plastified muscles; W – high pressure injector; N – low pressure injector; t – triceps muscle of arm; p – semimembranosus muscle.

Fig. 3. Value changes of relaxation times T_1 in the muscles examined

It was found that raw muscles were characterized by very similar values of relaxation times T_1: 610 ms for the *m. triceps brachii* and 621 ms – for the *m. semimembranosus*. In both muscles, the application of the low-pressure injector led to an increase in T_1 values as a result of the increased quantities of water in the system. Changes in the assessed parameters were slight and amounted to, respectively: 640 ms for the triceps muscle of the arm and 645 ms – for the semimembranosus muscle. The use of the spray injector caused a greater increase of T_1 values in the case of the semimembranosus muscle (665 ms), despite the fact that the quantity of water supplied to the system was identical. In the case of the *m. triceps brachii*, the T_1 value was observed to decline, even in comparison with the raw muscle (593 ms), implying good binding of the water introduced into the system. The plasticization process exposed organisational diversification of water in the examined muscles. Massaging of the triceps muscle of the arm further increased the T_1 value: to 688 ms after the low-pressure injector was applied, and to 719 ms when the spray injector was used. This phenomenon should be interpreted as an increase of free water content in the system. In the semimembranosus muscle samples after plasticization, the T_1 value decreased slightly in comparison with the samples analysed directly after the brine injection. This corresponded to a small decrease in the amount of free water in the examined muscle.

Analysing relaxation time values obtained from measurements, it can be concluded that the application of the low-pressure injector in the experiments led

to an increase in the relaxation time value T_1 in samples after injections and after plasticization. This was associated with the increase of the free water content in the system. This effect was observed in both of the examined muscles. It was found that the application of the high-pressure injector resulted in good water binding after the injection in the *m. triceps brachii*. However, after the plasticization process, there was an abundance of free water in the system. In the case of the *m. semimembranosus*, a significant drop in the content of free water counterbalanced the increase in the amount of free water after the injection following the plasticization process.

CONCLUSIONS

1. The results obtained indicate that, in each phase of the experiment, both the water content and the pH value as well as the *S/W* quotient depend on the type of muscle.

2. The water activity in the triceps muscle of the arm depends on the phase of the experiment, whereas in the semimembranosus muscle – on the curing technique.

3. The use of different curing techniques leads to differences in the amount of free drip as well as the pH value and the *B/W* quotient in the examined muscles.

4. The dynamics of the changes in the relaxation time value T_1 depends on the type of the cured muscle and on the brine injection technique applied.

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ANALIZA WŁAŚCIWOŚCI WODY W WYBRANYCH MIĘŚNIACH ŚWIŃ, PODDANYCH PROCESOWI PEKLOWANIA NASTRZYKOWEGO

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Streszczenie. W prezentowanej pracy przedstawiono wyniki badań, przeprowadzonych na dwóch mięśniach świńskich: trójgłowym ramienia *(musculus triceps brachii)* i półbłoniastym *(musculus semimembranosus)*. Ocenie poddano mięśnie surowe, po nastrzyku solanką pod zróżnicowanym ciśnieniem oraz po plastyfikacji. Na podstawie uzyskanych wyników stwierdzono zróżnicowaną zawartość wody oraz różne wartości pH i ilorazu zawartości chlorku sodu i wody (S/W) w obu rodzajach mięśni w każdej z faz doświadczenia. Zastosowanie odmiennych technik nastrzyku mięśni solanką peklującą (niskociśnieniowej i rozpyłowej) wpływało na ilość wycieku swobodnego z mięśni po nastrzyku oraz na wartość ilorazu B/W (stosunek zawartości białka i wody). Wzrost aktywności wody w mięśniu trójgłowym ramienia zaobserwowano dopiero po procesie plastyfikacji, natomiast w mięśniu półbłoniastym już po nastrzyku solanką peklującą. Wykorzystanie techniki opartej na zjawisku MRJ (Magnetyczny Rezonans Jądrowy) pozwoliło na stwierdzenia różnic w organizacji wody w obu analizowanych mięśniach w kolejnych fazach doświadczenia mimo, że nie stwierdzono ich między mięśniami surowymi.

Słowa kluczowe: mięśnie, techniki peklowania, właściwości wody, MRJ