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ANALYSIS OF WATER CONTENT AND DYNAMIC PROPERTIES IN MODEL STUDIES ON SELECTED PORCINE MUSCLES

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Abstract. The paper presents an analysis of the effect of curing brine composition on the water properties in sediments from pork shank and semi-membranous muscles. The applied brines were solutions of inorganic salts of varying composition. The performed analyses comprised active acidity pH, water activity A_w, water absorbability expressed as percentage proportion of free water in the total water content, as well as the value of relaxation times spin-network T_1 and spin-spin T_2. An inversely proportional correlation was found between pH values and water activity in the examined muscles when brines containing sodium chloride, pyrophosphate, tripolyphosphate and a mixture of NaCl and pyrophosphate were applied. In the case of the control muscles as well as those containing a mixture of sodium chloride and tripolyphoshate, the above correlation was directly proportional. It was demonstrated that pyrophosphate, even at low pH values, significantly increased water absorbability of muscle tissue sediments. The analysis of relaxation parameters showed that pyrophosphate as a constituent of brine reduced the content of free water in relation to bound water as demonstrated by significant shortening of the spin-network relaxation time. In addition, the molecular dynamics of bound water was found to be inhibited. The above observations allowed the authors to conclude that pyrophosphate, as brine constituent, causes very good water binding in muscle tissue sediments.

Keywords: water activity, water holding capacity, relaxation times

INTRODUCTION

Meat water holding capacity exerts a decisive influence on the quality of cured products manufactured from whole muscles and subjected to thermal processing (Rauusunen and Puolanne 2005). The introduction into meat components of curing mixtures, including sodium chloride and polyphosphates, improves their tenderness and decreases thermal drip from muscles during their thermal processing (Mc Gee *et al*. 2003), affects the aroma, texture and shelf life of the obtained products (Aktas *et al*. 2003), and regulates water activity (Rauusunen and Puo-

lanne 2005). The muscle structure is made up of myofibrillar proteins in amounts ranging from 55 to 60% and connective tissue – about 2-6% in relation to the total protein content in meat. The final desirable production results can be treated as a resultant of meat raw material quality, its tissue structure, as well as the quantity of the applied functional additives (Pospiech *et al*. 2003). Sodium chloride increases solubility of muscular proteins, enhances tissue water binding capacity, and improves the environmental ionic strength (Xargaýo *et al*. 1998; Medyński *et al*. 2000; Puolanna *et al*. 2001). After slaughter, the muscle pH value decreases and the myofibril filament structure contracts. This causes that most water occurs in the area of the sarcoplasm, i.e. inside the cell but outside myofilaments and then, gradually, gets outside as extracellular water. This confirms that the myofilament structure is also responsible for the capability of meat to retain meat juice. Nevertheless, the gradual loosening of the exceptionally hierarchical network system extending along and across muscle structure in rigor mortis which takes place during ripening also affects the water binding capacity. The dissociated NaCl ions form complexes with protein, giving it a different electrical charge, and favour dissociation of carboxyl groups changing the charge of protein molecules (Uchman 1998). The combined application of sodium chloride and phosphates increases considerably the degree of water binding in comparison with their separate application. Phosphates increase the ionic strength of the environment and exert a specific influence on myofibrillar proteins. They exert a similar influence to that of the adenosine triphosphate (ATP) in a living organism, i.e. they result in dissociation of the actomyosin complex. Myofibrillar proteins swell easier and pass into soluble state faster, which leads to improvement of water binding in the product. It should be emphasised that only phosphates are capable of "opening the structure", in other words, of dissociating actomyosin and exposing chemical bonds of muscular proteins of hydrophilic character, in contrast to the salts of other food acids which cause the swelling of meat proteins. Connective tissue proteins, collagen in particular, are also found to play a considerable influence by stabilising the protein-water system in meat products and affecting the texture of the final product (Nakamura *et al*. 2003, Urry and Parker 2002). The amount of collagen in individual culinary elements of the porcine carcass varies. Earlier investigations (Gajewska, unpublished) revealed that shank muscles are characterised by approximately 2.5-fold lower intramuscular collagen content than semi-membranous muscle. During thermal processing of meat products, collagen swells and is capable of binding large quantities of water (Szaciło and Cierach 2005).

Water binding capacity is one of the major characteristics of fresh meat quality (Andersen 2000, Honikel 1998). However, mechanisms affecting water holding capacity have not been recognised properly yet. In order to know them better, it is necessary to investigate characteristics of the types of water in the system and their

mutual relationships. This possibility has been made available by the low field nuclear magnetic resonance (NMR) technique (Baranowska *et al*. 2006a, Baranowska *et al*. 2006b, Bertram *et al*. 2001, Ruiz-Cabrera *et al*. 2004, Sorland *et al*. 2004).

The aim of the undertaken investigations was to compare water properties and its content in selected culinary elements of porcine carcasses. This study presents research results concerning the effect of the composition of curing brine on selected parameters characterising water properties in pork semi-membranous and shank muscles.

MATERIAL AND METHODS

Material

The experimental material comprised: pork semi-membranous muscle (P) and shank muscles (G) collected from swine carcasses 48 hours after slaughter.

The experimental muscles, following the removal of the epimysium and tendon elements, were subjected to two-time grinding through a grinder plate with 3 mm diameter mesh and mixed thoroughly. Samples prepared in this way were then divided into seven parts and each of these parts was subjected to the action of solutions of selected salts of clearly defined chemical composition.

The brines used in the performed experiments were prepared by dissolving salts of inorganic acids: sodium chloride (NaCl), disodium pyrophosphate ($Na_2H_2P_2O_7 \cdot 6H_2O$), sodium tripolyphoshate ($Na_5P_3O_{10}$) in deionised water at the temperature of 4-7^{o}C (Gajewska-Szczerbal *et al*. 2007). The quantity of the added salts was calculated in relation to the total muscle weight and water at the ratio of 1:2. Salt concentrations amounted to, respectively: 2% sodium chloride and 0.3% phosphates (converted into P_2O_5) (Gajewska-Szczerbal *et al*. 2007; Uchman 1998). In addition, samples were also prepared with the addition of brines containing combinations of sodium chloride and phosphates. The reference (control) samples in the discussed experiments were raw meat samples (S) and muscle tissue sediment in which brine was substituted by deionised water (W). Samples containing the solution of sodium chloride were designated as N, those containing the solution of pyrophosphate as P, and those containing the solution of tripolyphosphate as T. The muscle tissue sediments with the addition of brines composed of mixtures of sodium chloride and phosphates were designated as: P+N (samples containing the mixture of sodium chloride and pyrophosphate) and T+N (those containing the mixture of sodium chloride and tripolyphosphate). Samples of ground muscles were shaken in a shaker for 3 hours and then centrifuged for 5 minutes at 3000 rpm. This allowed obtaining a supernatant and sediment. The ultimate experimental material was the obtained muscular tissue sediment treated as a model system.

Measurement methods

The pH value was determined using the Accumet-15 pH meter against three buffer standards and employing a combined electrode. Water activity was measured with the assistance of the Aquaspector-1 apparatus with 0.005 accuracy after achieving relative air humidity equilibrium in the measuring chamber over the examined sample. The total water content in muscles, using the drying method at the temperature of 105°C, and the content of free water by the Grau-Hamm (Grau and Hamm 1957) method were assessed at each stage of the experiment.

The content of sodium chloride was determined by the Mohr method using silver nitrate (PN–73/A/ 82112/ Az 1 2002), while the content of phosphate was determined using the Quimociac reagent following muscle sample dry mineralisation (PN–ISO 13730 1999). Results of the phosphates analysis in the samples were given after conversion into P_2O_5.

Measurements of the spin-network T_1 and spin-spin T_2 relaxation times were carried out using a pulse NMR spectrometer operating at 30 MHz frequency with an inversion-recovery pulse sequence (measurements of the spin-lattice T_1 relaxation times) (Fukushima and Roeder 1981) and the sequence of CPMG (measurements of the spin-spin T_2 relaxation times) (Carr and Purcell 1954; Meiboom and Gill 1958). Measurements were conducted at the temperature of +20°C.

The results obtained during the performed experiments were subjected to triple-factor 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).

RESULTS AND DISCUSSION

In comparison with the control muscle samples, a considerable increase in the content of sodium chloride and phosphates was recorded in those samples of muscle tissue sediments which contained the addition of salts or their combinations. The same results were found for both parts of the *musculus longissimus dorsi* (Gajewska-Szczerbal *et al*. 2007).

Changes in the active acidity in the examined systems are presented in Figure 1. Shank muscles, both raw ones and those with the addition of water, were characterised by a higher pH value in comparison with the semi-membranous muscle. The composition of the applied brine was observed to influence changes of this parameter in both muscles. The addition of brines containing sodium chloride and tripolyphosphate decreased the acidity of the system in comparison with the control samples. The highest pH values were recorded in the shank muscles with the addition of tripolyphosphate and in the semi-membranous muscle with the addition of sodium

chloride. The mixture of these two compounds reduced the pH value. Identical results were obtained using the mixture of sodium chloride and tripolyphosphate. The lowest pH values were recorded when the applied brine contained pyrophosphate.

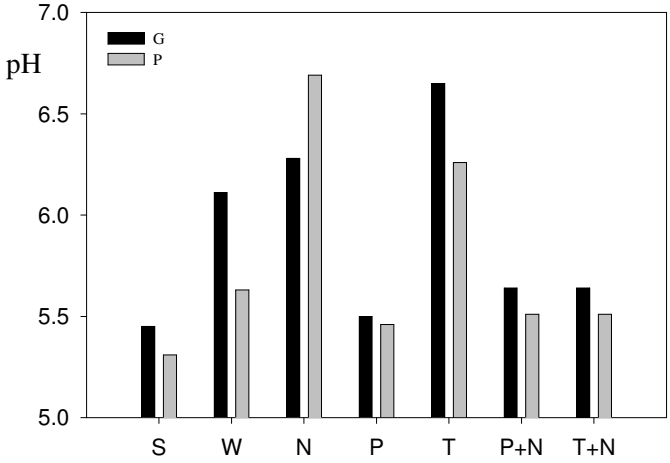

Fig. 1. Changes in the active acidity in the examined muscle sediments (G – shank muscles; P – semi-membranous muscle; S – raw sample, W – sample with water, N – sample with sodium chlorine, P – sample with tetrasodium diphosphate, T – sample with sodium triphosphate, P+N – sample with sodium chlorine and tetrasodium diphosphate, N+T – sample with sodium chlorine and sodium triphosphate)

The impact of the applied brines on changes in the value of water activity is shown in Figure 2.

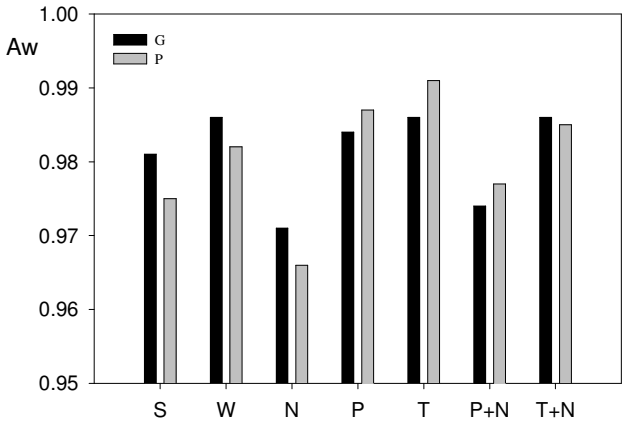

Fig. 2. Water activity (A_w) in the examined muscle tissue sediments (G – shank muscles; P – semi-membranous muscle; S – raw sample, W – sample with water, N – sample with sodium chlorine, P – sample with tetrasodium diphosphate, T – sample with sodium triphosphate, P+N – sample with sodium chlorine and tetrasodium diphosphate, N+T– sample with sodium chlorine and sodium triphosphate).

Both types of examined muscles were characterised by different water activity values. This parameter was found to increase in the systems containing deionised water in comparison with raw muscles. A_w values in the shank muscles were higher than in the semi-membranous muscle. Brines containing pyrophosphate and tripolyphosphate as well as the mixture of tripolyphosphate and sodium chloride were found to increase the value of this parameter. In comparison with the control samples, water activity declined in the systems containing brines with sodium chloride and the mixture of sodium chloride and pyrophosphate.

The capacity of the system to retain water is indicated by the value of the percentage proportion of free water in the total water content in the system. Changes in this parameter, numerically corresponding to water holding capacity, are presented in Figure 3.

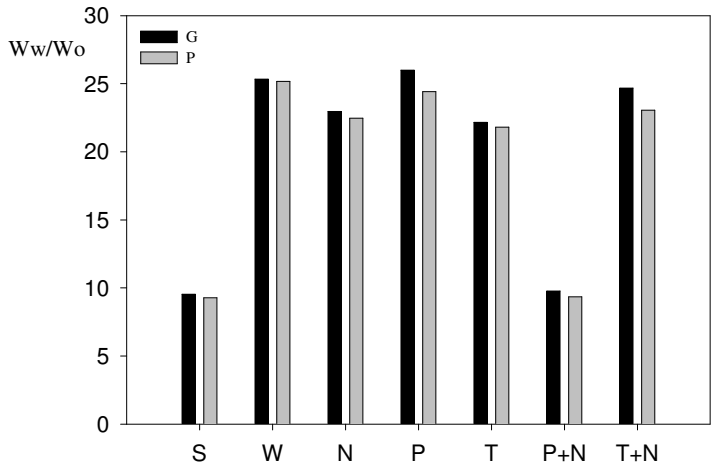

Fig. 3. Percentage proportion of free water (W_w) in the total water content (W_o) in the examined muscle sediments (G – shank muscles; P – semi-membranous muscle; S – raw sample, W – sample with water, N – sample with sodium chlorine, P – sample with tetrasodium diphosphate, T – sample with sodium triphosphate, P+N– sample with sodium chlorine and tetrasodium diphosphate, N+T – sample with sodium chlorine and sodium triphosphate)

No differences were found in the water holding capacity of the examined muscle samples. In comparison with the sample containing water, a slight increase in water binding capacity was observed when brines with pyrophosphate, tripolyphosphate, sodium chloride and the mixture of the last two compounds were applied. A distinct decrease in the water binding capacity of the system was observed only when brines containing the mixture of sodium chloride and pyrophosphate were employed.

The results above presented indicate that the water holding capacity – the parameter which should characterise the possibility of retaining water in the muscle tissue sediment – failed to indicate clearly which of the applied curing brines guaranteed the best results in the examined muscles. Similar results were obtained analysing the influence of these brines on the water holding capacity of the *musculus longissimus dorsi* (Gajewska-Szczerbal *et al*. 2007).

In order to supplement the above results, the authors carried out an analysis of changes in the values of spin-lattice T_1 and spin-spin T_2 relaxation times in the examined muscle tissue sediments. This allowed detailed monitoring of changes in the mutual relationships between free and bound water as well as of the molecular dynamics of the two water sub-systems on the molecular level.

Value changes in the spin-lattice relaxation time in the examined muscle tissue sediments are shown in Figure 4. As in the case of the analysis of the pH and A_w values, also here differences were found between raw muscles and water containing systems. The T_1 value increased in all the systems with the exception of those in which brine with pyrophosphate was applied.

In all analysed cases, it was found that shank muscles were characterised by longer T_1 relaxation time than the semi-membranous muscle. The longer time of relaxation indicates a higher content of free water in relation to bound water.

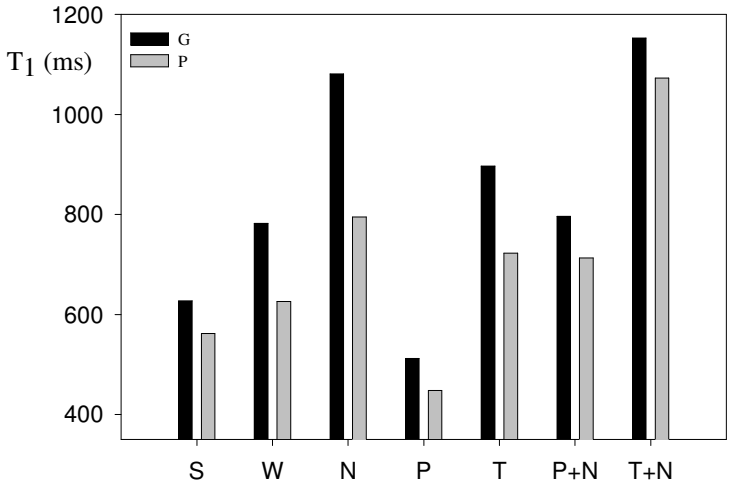

Fig. 4. Value changes in the spin-lattice relaxation time (T_1) in the examined muscle tissue sediments (G – shank muscles; P – semi-membranous muscle; S – raw sample, W – sample with water, N – sample with sodium chlorine, P– sample with tetrasodium diphosphate, T– sample with sodium triphosphate, P+N – sample with sodium chlorine and tetrasodium diphosphate, N+T – sample with sodium chlorine and sodium triphosphate)

Figures 5 and 6 present value changes in the short – T_{21} and long – T_{22} component of the spin-spin relaxation time.

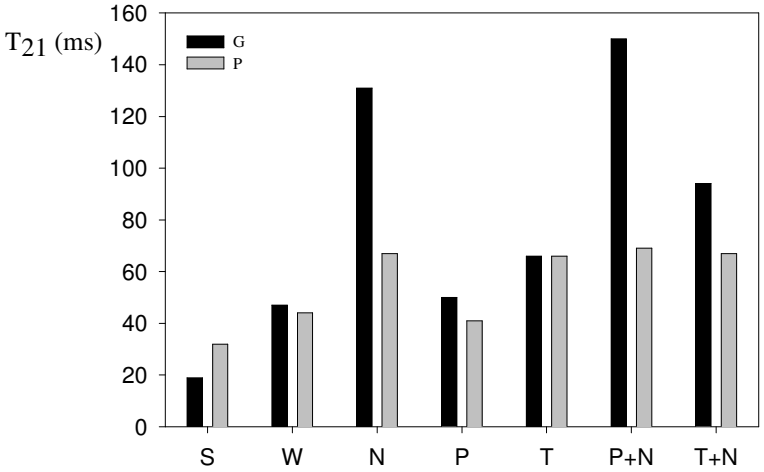

Fig. 5. Value of the short component of the spin-spin relaxation times in the examined muscle tissue sediments (G – shank muscles; P – semi-membranous muscle; S – raw sample, W – sample with water, N – sample with sodium chlorine, P – sample with tetrasodium diphosphate, T – sample with sodium triphosphate, P+N – sample with sodium chlorine and tetrasodium diphosphate, N+T – sample with sodium chlorine and sodium triphosphate)

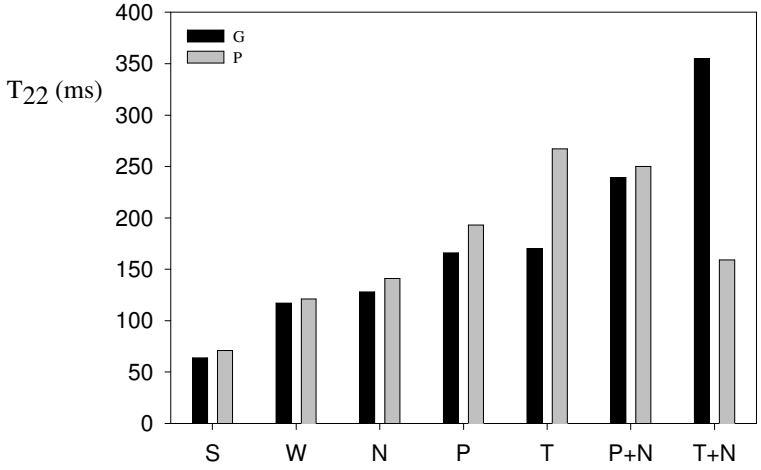

Fig. 6. Value of the long component of the spin-spin relaxation times in the examined muscle tissue sediments (G – shank muscles; P – semi-membranous muscle; S – raw sample, W – sample with water, N – sample with sodium chlorine, P – sample with tetrasodium diphosphate, T– sample with sodium triphosphate, P+N – sample with sodium chlorine and tetrasodium diphosphate, N+T– sample with sodium chlorine and sodium triphosphate)

The addition of deionised water to the examined muscles resulted in levelling out in the values of the relaxation time two components in the examined muscles. The free water fraction was characterised by a greater value than in the systems with water molecular dynamics. The highest T_{22} values were observed when the T+N brine was applied in the case of the shank muscles and the T brine – in the case of the semi-membranous muscle. No differences were recorded in the T_{22} values of the relaxation time in the case of the semi-membranous muscle containing N, T, T+N and P+N brines. In comparison with the control sample W, the pyrophosphate-containing brine failed to change the T_{21} value. On the other hand, shank muscles showed different T_{21} values, depending on the applied brine. Sodium chloride and the mixture of NaCl with tripolyphosphate and with pyrophosphate led to a significant increase in the T_{21} values.

The above-presented analysis of the obtained results indicates unequivocally that the percentage share of free water in the total water content cannot be treated as a parameter which allows unambiguous assessment of the water binding capacity in muscle systems. The lowest free water content in the total water content was recorded in the case of the mixture of NaCl and pyrophosphate as brine components. However, brines which contained these compounds separately failed to exhibit significant changes in this parameter in comparison with the control sample. The remaining analysed parameters were found to be different in both of the examined muscles. The differences between the examined muscles were most apparent in the case of relaxation time investigations. Shank muscles characterised by longer spin-network relaxation times contained higher quantities of free water in relation to bound water. In addition, with the exception of the application of the NaCl-containing brine, the above muscles showed higher pH values in comparison with the semi-membranous muscle. Despite the fact that in the raw shank muscles bound water was less mobile than in the semi-membranous muscle, the addition to the system of both deionised water and brines caused a considerably higher inactivation of the molecular motions of this water fraction. The research results obtained using the NMR technique showed that, in the case of the semi-membranous muscle, water after the addition of brine was bound much better than in the case of the shank muscles. The above observations corroborate the need to supplement classical research methods by relaxation measurements in order to obtain a more comprehensive description of the state of water in muscle systems. It is possible then to obtain more precise information about both the quantitative and qualitative relationships between the free and bound water in the examined system.

CONCLUSIONS

1. An inversely proportional relation was found between pH values and water activity in the examined muscles employing brines containing sodium chloride,

pyrophosphate, tripolyphosphate and the mixture of NaCl and pyrophosphate. This relation was directly proportional in the control muscles as well as in muscles containing the mixture of sodium chloride and tripolyphosphate.

2. Pyrophosphate, even at low pH values, was found to increase significantly the water holding capacity of muscle tissue sediments. A similar action was observed when the applied curing brine was a mixture of tripolyphosphate and sodium chloride. This type of brine composition is already used to cure this kind of meat.

3. The performed analysis of relaxation parameters revealed that pyrophosphate used as a component of the brine reduced the free water content in relation to bound water as evidenced by a considerable shortening of the spin-lattice relaxation time. In addition, the molecular dynamics of bound water was found to be inhibited. The above observations allowed the authors to conclude that pyrophosphate used as brine component caused very good water binding in muscular tissue sediments.

4. The use of brines containing sodium chloride led to very poor water binding in shank muscles as confirmed by relaxation studies. In these systems, significant value shortening of the short component of the spin-spin relaxation time was observed at a simultaneous lengthening of the spin-network time. The semimembranous muscle showed a considerably weaker water liberation when the above-mentioned brines were employed.

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ANALIZA ZAWARTOŚCI I WŁAŚCIWOŚCI DYNAMICZNYCH WODY W MODELOWYCH BADANIACH WYBRANYCH MIĘŚNI ŚWIŃ

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S t r e s z c z e n i e . W prezentowanej pracy analizowano wpływ składników solanek peklujących na właściwości wody w osadach mięśni golonki i mięśnia półbłoniastego. Składnikami solanek były roztwory soli nieorganicznych o zróżnicowanym składzie. Analizowano kwasowość czynną pH, aktywność wody A_w, wodochłonność wyrażoną jako procentowy udział wody wolnej w ogólnej zawartości wody oraz wartości czasów relaksacji spin-sieć T_1 i spin-spin T_2. Stwierdzono odwrotnie proporcjonalną zależność pomiędzy wartościami pH i aktywnością wody w badanych mięśniach, przy zastosowaniu solanek zawierających chlorek sodu, pirofosforan, trójpolifosforan i mieszaninę NaCl i pirofosforanu. W mięśniach kontrolnych i zawierających mieszaninę chlorku sodu i trójpolifosforanu zależność ta jest wprost proporcjonalna. Wykazano, że pirofosforan, nawet przy niskich wartościach pH znacząca podwyższa wodochłonność osadów mięśniowych. Analiza parametrów relaksacyjnych wykazała, że pirofosforan jako składnik solanki obniża zawartość wody wolnej w stosunku do wody związanej, co przejawia się znacznym skróceniem czasu relaksacji spin-sieć. Dodatkowo zaobserwowano zahamowanie dynamiki molekularnej wody związanej. Powyższe pozwala wnioskować, że w osadach mięśniowych pirofosforan jako składnik solanki powoduje bardzo dobre wiązanie wody.

S ł o w a k l u c z o w e : aktywność wody, czasy relaksacji, wodochłonność