THE INFLUENCE OF SARCOPLASMIC PROTEIN DENATURATION ON THE MYOWATER DISTRIBUTION AND MOBILITY: A LOW-FIELD NUCLEAR MAGNETIC RESONANCE STUDY

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Abstract – It was recently proposed that in PSE-like condition denatured sarcoplasmic proteins influence the water population within different compartments (intramyofibrillar, intermyofibrillar and extracellular spaces) differently. In the present study, muscle strips with and without sarcoplasmic proteins were incubated at 44 ˚C to induce protein denaturation. The impact of denatured sarcoplasmic proteins on the water distribution and mobility was investigated by low-field nuclear magnetic resonance (LF-NMR) relaxation measurement. T_2 relaxation data was analysed using biexponential fitting. PSE-like protein denaturation induced translocation of myowater from inside myofibrils to the extra-myofibrillar space (intermyofibrillar and extracellular spaces). Shorter relaxation time of T_{21} and T_{22} suggested that denatured sarcoplasmic proteins enhanced the interaction between water and protein matrix both in intramyofibrillar space and the space outside of myofibrils.

Key Words – Water-holding capacity; Protein network

I. INTRODUCTION

Protein denaturation occurs both in the sarcoplasmic and myofibrillar protein fractions in pale, soft and exudative (PSE) meat, which is believed to be the reason for the loss of water-holding capacity as reviewed in [1]. Recently a new hypothesis was proposed by Liu, Arner, Puolanne and Ertbjerg [2] stressing that in PSE condition sarcoplasmic proteins precipitate and coagulate both within and outside of myofibrils, having an overall positive impact on water-holding capacity. The process was proposed to be compartmentalized: 1) between thick and thin filaments, sarcoplasmic proteins precipitate in parallel with lateral shrinkage of the lattice, which pushes the water out of the myofibrils; 2) outside of myofibrils, denatured sarcoplasmic proteins form a protein network, which traps the water that has migrated out.

Low field (LF)-NMR relaxometry is a non-invasive technique that has been used to study the water distribution and mobility within the muscle matrix. Transverse relaxation (T_2) of water can be decomposed into two or three exponential components. Based on biexponential analysis, the fast-relaxing water population (P_{21}) occupies around 80-95% of the total population and is characterized by a relaxation time of 35-50 ms (T_{21}) while the slower-relaxing population (P_{22}), i.e. characterized by a relaxation time of 100-250 ms (T_{22}), and occupies 5-15% of the total population [3]. The water population P_{21} corresponds to the water molecules held within the myofibrillar protein matrix i.e. mainly between filaments. The slower relaxing population P_{22} corresponds to the water outside of myofibrils [3]. The T_2 time constants reflect the mobility of the water molecules held within each compartment and therefore they are often used to imply structural alterations within the myofibrillar protein matrix as well as in the space outside of myofibrils [4].

The aim of the current study is to investigate the influence of denatured sarcoplasmic proteins on the water population and mobility in the intra- and extra-myofibrillar space. To achieve this, LF-NMR measurements were done on skinned porcine *longissimus thoracis et lumborum* (LTL) muscle strips after they were temperature incubated at 21 and 44 ˚C with and without sarcoplasmic proteins.

II. MATERIALS AND METHODS

2.1 Raw materials

Three LTL muscles were sampled at 24 h post mortem from different pig carcasses of the cross Norwegian Landrace x Swedish Yorkshire x Danish Landrace. From each muscle 12 muscle strips (5 x 5 x 40 mm) were cut along the fibre direction and then chemically skinned by removing the sarcolemma in a rigor solution with detergent at 4 ˚C for 24 h according to [2]. Sarcoplasm and protein-depleted sarcoplasm were prepared as described in [2].

2.2 Treatments

Skinned muscle strips were incubated in sarcoplasm (pH 5.5) and protein-depleted sarcoplasm (pH 5.6) for 24 h at 4 ˚C in order to bring back sarcoplasm with or without sarcoplasmic proteins. Thereafter, the muscle strips were incubated at 21 and 44 ˚C for 1 h. After incubation, the muscle strips were gently cleaned on the surface by soft tissue paper and thereafter kept in a storage buffer at pH 5.5 mixed 1:1 (v : v) with glycerol [2] overnight at 4 °C allowing full diffusion. The next day these strips in the same buffer were moved to -20 ˚C for storage until LF-NMR measurements. Four different treatments (temperature incubation of strips at 21 and 44 ˚C with or without sarcoplasmic protein) were done and in each treatment, triplicates were done from each LTL muscle.

2.3 LF-NMR relaxation measurements

Muscle strips were removed from the storage buffer and gently rolled on a piece of tissue paper to remove excessive moisture on the surface.

The LF-^{1}H-NMR measurements were performed on a Maran Ultra spectrometer (Resonance Instruments, UK) operating at a Larmor frequency of 23.2 MHz for ^{1}H using a probe equipped for 18 mm (o.d.) sample tubes. Transverse relaxation T_2 were recorded using the Carr-Purcell-meiboom-Gill (CPMG) pulse sequence [5] employing a recycle delay of 4 s, a τ-delay of 100 μs and 16 scans for recording of 8000 echo maxima at 25 ˚C. The biexponential fitting analysis of T_2 data was done according to [6].

2.4 Statistical analysis

Two way analysis of variance (ANOVA) was done by JMP® 9.0.0 (JMP, Cary, NC) using the Fit Model platform with temperatures and the presence of sarcoplasmic proteins as the main effects. Animal number was defined as a random factor. Least square means were compared by Tukey's HSD (honest significant difference) test using pairwise comparison method using $P < 0.05$ for significance.

III. RESULTS

As shown in Table 1, temperature incubation had significant influences on the variables T21, T22, P21 and P22. The presence of sarcoplasmic proteins during incubation had significant influences on T21, P21 and P22.

As shown in Table 2, T_{21} and T_{22} time constants were slightly higher compared to the earlier observations at 24 h post mortem [7]. In general incubation at 44 ˚C decreased the T_{21} and T_{22} relaxation times compared to 21 ˚C. The presence of sarcoplasmic proteins reduced the T_{21} at both incubation temperatures, whereas the effect on T_{22} was only significant when comparing the presence of sarcoplasmic protein at 21 and 44 ˚C.

Table 1. Significance level of main effects and their cross-effects on T_{21}, T_{22}, P_{21} and P_{22} from biexponential analysis of CPMG (Carr-Purcell-meiboom-Gill) data measured from skinned strips of porcine LTL muscles temperature incubated with or without added sarcoplasmic proteins (SP).

Variables	SP	Temperature	cross-effect
$\mathbf{T_{21}}$	< 0.001	< 0.001	0.008
$\mathbf{T_{22}}$	0.2	0.007	0.02
$\mathbf{P_{21}}$	0.02	< 0.001	0.04
$\mathbf{P_{22}}$	0.02	< 0.001	0.04

Table 2. Time constants of components T_{21} and T_{22} of skinned strips of porcine LTL muscles temperature incubated with or without added sarcoplasmic proteins (SP).

T_2 relaxation time constant (ms)	21 ˚C		44 ˚C	
	-SP	+SP	-SP	+SP
T_{21}	61[a]	58[b]	58[b]	51[c]
T_{22}	300[ab]	320[a]	296[ab]	260[b]

Within each row [abc]Least Square means with same letter do not differ (P > 0.05).

The combination of the water populations P_{21} and P_{22} equal to 100% and therefore only P_{22} values are presented here. P_{22} represents the extramyofibrillar water, which increased significantly in the group with PSE-like denaturation of sarcoplasmic and myofibrillar proteins (Figure 1).

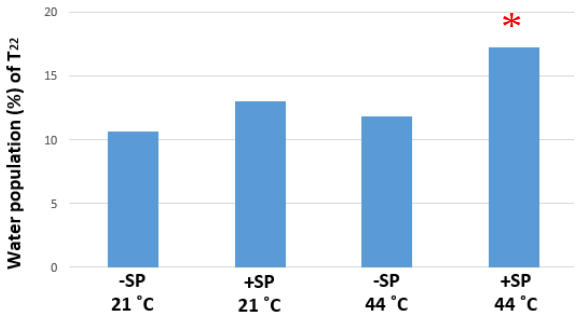

Figure 1. Water population representing T_{22} component from biexponential analysis of CPMG data measured from skinned strips of porcine LTL muscles temperature incubated with or without added sarcoplasmic proteins (SP). +SP, 44 ˚C is significantly higher than the rest of results. Standard error: 0.9%.

IV. DISCUSSION

Our set-up of post-rigor incubation of pork at 44 ˚C at pH 5.5-5.6 is able to induce PSE-like characteristics including poor water-holding of myofibrils and denaturation of sarcoplasmic and the myofibrillar proteins [2].

P_{22} represents the amount of water held in extramyofibrillar space and this water population is more susceptible to be lost as drip after slaughter [3]. In the treated samples designed to mimic the PSE condition (+SP, 44 ˚C) higher P_{22} values were obtained compared to "normal meat" (+SP, 21 ˚C), which is in agreement with the previous observation in beef with rapid pH decline [7]. This difference in P_{22} indicates that in accompany with protein denaturation in the PSE condition, more myowater is expelled from the intra- to the extra-myofibrillar space due to lateral shrinkage of the filament lattice. This speculation is also supported by the previous study [2], showing that heat-denatured sarcoplasmic protein shrank the lattice spacing of skinned muscle fiber bundles, and therefore enlarged extracellular space correspondingly. However, in other studies [1] and [8], no differences were found in neither P_{21} nor P_{22} in comparisons between RSE (red, soft and exudative) *vs* normal meat and PSE vs normal meat.

PSE meat has previously been reported to have shorter T_{21} relaxation time [8] and according to [7] in beef with rapid pH decline, T_{21} was also shorter than the ones with normal pH decline rate. This is in line with our observation that temperature incubation reduced the T_{21} relaxation time (Table 2).

An increase in T_{21} reflects less strong binding of water by the protein matrix inside of myofibrils, which previously has been suggested to be related to a lower protein concentration in the lattice due to the swelling of sarcomere, the leakage of sarcoplasm and stretching of sarcomere [3, 7]. The difference observed at 21 ˚C between +SP *vs* –SP (Table 2), suggests that the presence of sarcoplasmic proteins increases the protein density thus resulting in a lower T_{21}. More importantly, the denatured sarcoplasmic proteins reduced the T_{21} even more, i.e. 12% compared to 5 % by undenatured sarcoplasmic proteins. Two phenomena could account for this effect: 1) the lattice is more compressed by the denatured sarcoplasmic proteins as already shown by X-ray diffraction [2], and since the sarcomere lengths were the same among all the samples (results not shown), the protein density was higher; 2) the denatured sarcoplasmic proteins formed a network, which interacted

more strongly with water molecules than soluble sarcoplasmic proteins.

The T_{22} time constant was reported to be shorter in PSE compared to normal meat [8], which is in line with our results (+SP, 44 ˚C *vs* +SP, 21 ˚C). The impact of denatured sarcoplasmic proteins in the extramyofibrillar space is significant, as it reduced T_{22} level by 19%, indicating that the network of coagulated sarcoplasmic proteins in the extramyofibrillar space contributes to water-binding.

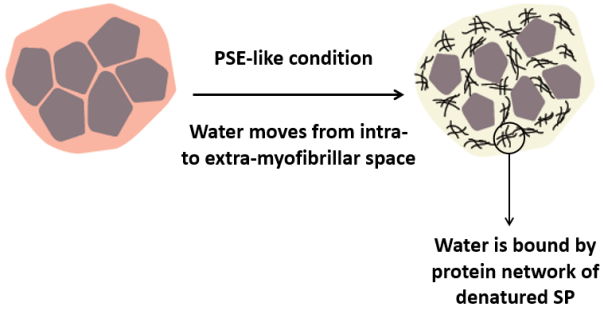

Figure 2. Illustration showing the change in water distribution and mobility during PSE-like denaturation at fibre bundle level. Note: the denaturation may also occur between and within myofibrils (inside of muscle fibres), which is not shown here. SP: sarcoplasmic protein.

The LF-NMR results provide additional information regarding the myowater population and mobility in compartments of intra- and extra-myofibrillar space in PSE meat. As illustrated in Figure 2, in the PSE-like condition, sarcoplasmic protein precipitation occurs in parallel with additional shrinkage of the lattice spacing and consequently causes re-distribution of the water population, i.e. pushing water from intra- to extra-myofibrillar space. However, when water reaches the extramyofibrillar space, it is trapped and held more tightly by the network formed by the coagulated sarcoplasmic proteins. Therefore, the overall water-holding capacity becomes better compared to the situation where no denatured sarcoplasmic proteins are present.

V. CONCLUSION

The current study supports the new hypothesis [2] that in PSE-like condition denatured sarcoplasmic proteins influence the water distribution and mobility in a compartmentalized way: 1) within the intramyofibrillar space, the denatured sarcoplasmic proteins shrinks the lattice and water is expelled; 2) within extramyofibrillar space, water is trapped by the protein network formed by coagulated sarcoplasmic proteins.

REFERENCES

1. Kim, Y. H. B., Warner, R. D., & Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. Animal Production Science, 54(4), 375–395.
2. Liu, J., Arner, A., Puolanne, E., & Ertbjerg, P. (2016). On the water-holding of myofibrils: Effect of sarcoplasmic protein denaturation. Meat Science, 119, 32-40.
3. Bertram, H. C., & Andersen, H. J. (2004). Applications of NMR in meat science. Annual reports on NMR spectroscopy, 53, 157-202.
4. Bertram, H. C., Purslow, P. P., & Andersen, H. J. (2002). Relationship between meat structure, water mobility, and distribution: A low-field nuclear magnetic resonance study. Journal of Agricultural and Food Chemistry, 50(4), 824-829.
5. Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear relaxation times. Review of scientific instruments, 29(8), 688-691.
6. Miklos, R., Cheong, L. Z., Xu, X., Lametsch, R., & Larsen, F. H. (2015). Water and fat mobility in myofibrillar protein gels explored by low-field NMR. Food Biophysics, 10(3), 316-323.
7. Tornberg, E., Wahlgren, M., Brøndum, J., & Engelsen, S. B. (2000). Pre-rigor conditions in beef under varying temperature-and pH-falls studied with rigometer, NMR and NIR. Food Chemistry, 69(4), 407-418.
8. Tornberg, E., Andersson, A., Göransson, Å., & Von Seth, G. (1993). Water and fat distribution in pork in relation to sensory properties. Pork quality: Genetic and metabolic factors, 239-256. CAB International: Wallingford.
9. Bertram, H. C., Karlsson, A. H., Rasmussen, M., Pedersen, O. D., Dønstrup, S., & Andersen, H. J. (2001). Origin of multiexponential T2 relaxation in muscle myowater. Journal of Agricultural and Food Chemistry, 49(6), 3092-3100.