The Effect of Low-Frequency and Intensity Ultrasound on Pre-Rigor Meat on Structure and Functional Parameters of Freezing and Thawed Beef Semimembranosus Muscle

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The quality of meat today is highly variable. This is one of the biggest problems in the beef industry. Tenderness and functional properties are the most important quality characteristics of meat. They are influenced both by numerous ante-mortem factors and by post-mortem processing methods. Meat tenderness is influenced mainly by changes in protein myofibrilar fraction and the connective tissue fraction (4,5,6). As many authors reported, changes in meat tenderness can be largely attributed to the activities of two basic enzyme groups in meat tissue, which modulate the protein substance. The first group are calpains, associated with the system and control of muscle contraction, the second one are lysosomal catepsines (3,5,6). This study has attempted to analyse the impact of post-mortem meat sonication on some of its structural physical and functional properties during the post-mortem cold storage (48 h) and on changes caused by meat freezing (thawed meat).

MATERIALS AND METHODS

Muscle samples. Muscle semimembranosus was removed 90 minutes post-mortem from cattle with ante-mortem weight of about 500 kg. It was cut into two sections of about 200 g each. The obtained samples served as research material prepared as follows. The prepared samples of meat tissue from 10 cattle items were divided into three parts (with 10 samples in each part) of which one was the control sample (K), another one was 2 h post mortem sonicated for 2 minutes with ultrasounds of 25 kHz frequency and 2 $W \cdot cm^{-2}$ volume of converter (U1). The third one was sonicated for time for 2 minutes 12 h post-mortem (U2). The samples were then stored at 6-8°C. After 48 h (2 days post-mortem) muscles were vacuum packed into plastic bags and frozen until –20°C. After 3 months of freezing the samples were being thawed for 24 h in room temperature (18°C) and the evaluated for change of structure, physical and functional properties. *Texture Profile Analysis* (1). The meat was cooked in assigned temperature and cubic samples (2 cm) were cut out of meat tissue. They were compressed twice until 50% of the initial height in the direction vertical to the muscle fibres. The results include values of hardness 1st and hardness 2nd. Testing machine INSTRON 4302 (crosshead speed 10 $mm \cdot min^{-1}$) was used for the compression. *pH*. The pH was determined from 2 g of sample (homogenised with 10 g water) on N5170 pH-meter provided with a universal electrode. *Shear force (tenderness)*. An Instron Universal Testing Machine model 4302 shear attachment and a crosshead speed of 10 $mm \cdot min^{-1}$ were used. The sample meat (1×1×4 cm) was cooked in a water bath 50, 60 and 70°C for 30 minutes. *Water Holding Capacity*. The Water Holding Capacity (WHC) was determined using duplicate 50 g samples homogenised in 50 ml water for 1 minute. Samples were then centrifuged at 5000·g at 6°C for 10 minutes. The following value was calculated as a measure of $WHC=(A-B)/C \times 100\%$, where: A – water addition to meat, B – weight of water after centrifugation, C – weight of sample. *Cook loss*. The weights of samples before and after cooking (in temperatures 50, 60 and 70°C) were recorded and the cook loss calculated for all replicates per treatment. *Thaw loss*. The weights of sample before and after freezing (thawed meat). *Histological studies*. Light Microscope. Samples (≈5 mm) were obtained and fixed in Bouin solution, dehydrated in ethanol solution and embedded in paraplast, stained with hematoxilin and eosin and observed with photomicroscope and photographed with Kodak TRi-X film at a magnification of ×100. Electron Microscope. Samples were cut and fixed in 2% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 2 h at room temperature, washed several times in buffer and fixed with 1% osmium tetroxide. Then these samples were dehydrated in a graded series of ethanol solutions and embedded in upon 812 mixture. Thin sections were made with an ultramicrotome (Reihart'am – U3) using a diamond knife and stained with 1% uranyl acetate dissolved in 50% ethanol and with undiluted lead citrate and observed with a Tesla 3S-500 electron microscope at a magnification of ×8000.

RESULTS AND DISCUSSION

The results of meat evaluation (using TPA method) presented in Table 1 for hardness 1st and hardness 2nd show that post-mortem ultrasound meat treatment changes its tenderness characteristics. Their examination after cooking at 50°C showed the highest hardness 1st value for sample (U) sonicated for 2 minutes. This phenomenon is observed for sample chilled for 48 hours post-mortem. After cooking at 70°C the highest hardness 1st value was shown for sample (U) after 24 h and sample (U1) after 48 h post-mortem. Shear forces (tenderness) show similar value change as in hardness 1st and 2nd after 48 hours of refrigerated storage.

The highest values of max. shear force (Table 2) for all the tested temperature are shown in sample (U1). Double ultrasound treatment causes significant drop in shear force value (U2), which nevertheless remain higher than in control sample. Shear force examination for samples cooked at 70°C shows that the longer period post-mortem (until 48 h) the higher shear force values in control sample: from 84.1 $N \cdot cm^{-2}$ after 48 h to 87.2 $N \cdot cm^{-2}$ after freezing, whereas in sonicated samples the values drop with the growing time of storage: for (U1) from 60 to 72,1 $N \cdot cm^{-2}$ and for (U2) from 69.5 to 75.2 $N \cdot cm^{-2}$.

The results of shear force examination show the highest difference in values for samples cooked at 50°C and the lowest for those cooked at 70°C. These results may point out that ultrasound meat treatment post mortem causes the greatest change in protein myofibril structure and a relatively smaller one in other protein fractions (collagen). This is also confirmed by the results for cooking loss. After 48 h post mortem cooking loss values at 80°C remain on the same level and after 24 h the lowest value is shown for control sample. This phenomenon may be caused by greater rigor mortis in sonicated samples.

Table 1. Ultrasound treatment influence on hardness 1st and 2nd (TPA) of beef semimembranosus muscle

Variable	Hardness [$N \cdot cm^{-2}$]	50°C			60°C			70°C		
		K	U1	U2	K	U1	U2	K	U1	U2
48 h post mortem	H_1	24.9±1.6	26.8±1.9	23.5±1.1	26.6±1.5	31.6±2.3	26.2±2.3	22.4±1.7	32.4±2.4	27.5±2.1
	H_2	22.7±1.1	23.3±0.6	22.0±0.9	24.9±0.9	18.6±2.0	24.3±1.9	20.5±1.3	29.7±1.8	24.7±1.0
unfrozen	H_1	26.5±1.5	26.2±1.6	17.1±2.9	28.1±3.1	24.4±2.4	31.1±2.6	32.1±2.6	34.1±2.3	33.1±2.7
	H_2	21.4±1.1	22.4±1.4	16.2±1.6	25.0±1.5	21.2±1.8	30.3±2.5	30.3±2.5	31.0±1.6	32.0±2.6

Table 2. Ultrasound treatment influence on cooking loss and tenderness (instrumental shear properties) of beef semimembranosus muscle

Variable		50°C			60°C			70°C		
		K	U1	U2	K	U1	U2	K	U1	U2
Shear force [$N \cdot cm^{-2}$]	48h p.mortem	74.1±5,6	64.0±6,2	68.4±3,6	86.1±6,4	68.5±6,2	72.0±4,6	84.1±6,2	70.0±5,3	69.5±7,2
	unfrozen	72,0±3.4	70,4±4.3	72,5±3.9	89,1±3.6	76,9±6.4	74,1±5.1	87,3±2.1	72,1±4.8	70,4±3.6
Cooking loss [%]	48h p.mortem	7.0±0,6	7.1±0,3	6.5±0,4	15.4±0,4	14.6±2,1	15.1±1,4	17.2±1,0	15.8±1,6	16.8±2,3
	unfrozen	8,2±1.1	7,3±0.9	7,1±0.7	18,8±2.1	15,9±1.4	16,6±0.8	23,2±2.1	21,6±1.4	18,8±2.1

Examination of thaw loss (Table 3) showed that ultrasound meat treatment caused drop in the loss value from 3% in control sample (K) to 2% in sample (U2). However, a small rise is observed in WHC of sonicated samples while pH value of thawed meat for evaluated samples remains on the same level: (K) – 5.60, (U1) – 5.75, (U2) – 5.75. The observed water holding capacity differences confirm the results for cooking loss value. Whereas the sonicated samples had lower thaw loss, during their cooking the loss also remains lower than in the control sample. Tenderness properties evaluation using the TPA method show that the sonicated samples (U1) and (U2) have the value levels for hardness 1st and 2nd similar to those in the control sample (K) (Table 1). Tenderness examination (Table 2) showed that the max. shear force is highest in the control sample for all the tested cooking temperatures. It is probably dependent on the value of cooking loss, which as it has already been stated, is highest for the control sample, and furthermore, drops in samples water content causes rise in its shear force value.

Table 3. Ultrasound treatment influence on WHC, thawed loss and pH of chilling and unfrozen beef semimembranosus muscle

Variable	WHC			pH			thawed loss [%]		
	K	U1	U2	K	U1	U2	K	U1	U2
48 h post mortem	23,0±2.4	26.5±3,6	27,4±2.8	5.55±0.1	5.80±0,1	5,85±0.1	–	–	–
unfrozen	19.0±2,0	24,2±2.0	25.4±2,3	5,60±0.1	5.75±0,1	5,75±0.1	3.0±0,1	3,0±0.1	2.0±0,1

Light and electron microscopy are powerful tools in the study of the microstructure of muscle. The examination of microstructure (Fig.1 and 2) have showed that ultrasounds have a destructive influence on morphological elements and structural degradation in myofibrilar proteins. Many myofibrils area seen to be broken in the area of the Z-discs, and I-bands and M-line have virtually disintegrated (Fig.2).

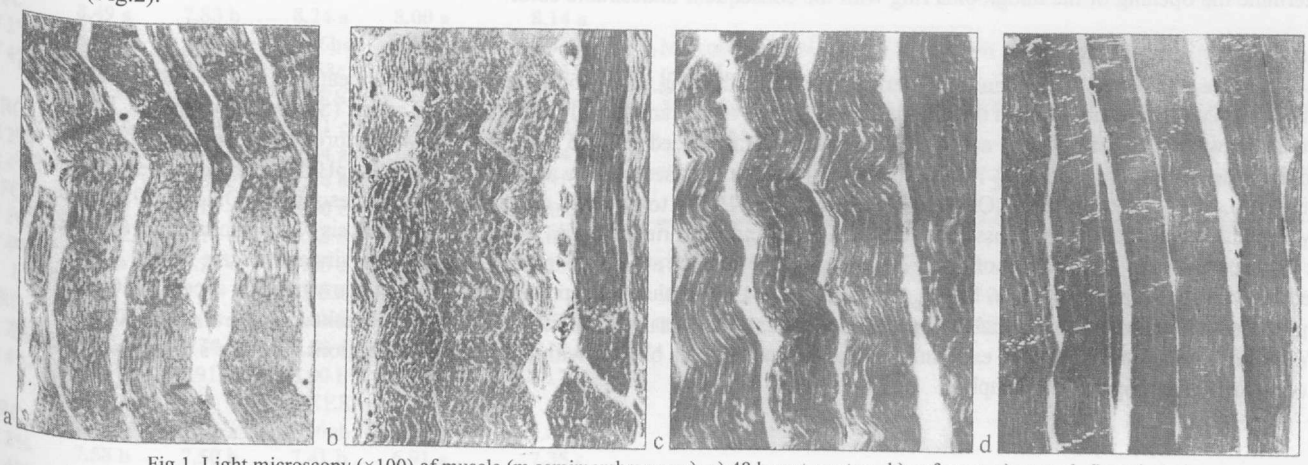

Fig.1. Light microscopy (×100) of muscle (m.semimembranosus): a) 48 h post mortem, b) unfrozen, c) control, d) sonicated

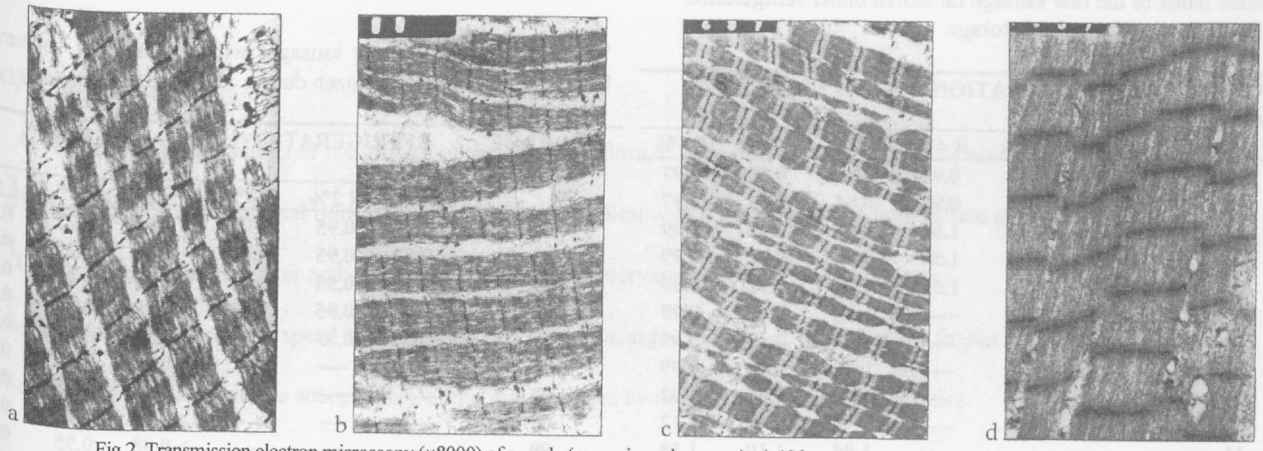

Fig.2. Transmission electron microscopy (×8000) of muscle (m.semimembranosus): a) 48 h post mortem, b) unfrozen, c) control, d) sonicated

Differences are observed in (U2) and control samples (K) (see Fig.0) conditions the myofibers. Were completely destroyed in (U) samples. We have in sonication samples after freezing completely destruction sarcomers. These differences are more clearly seen in Fig.2 where the border between A and I-bands is not clear (a lot of differences between U-samples and K-samples) and the Z-lines are to have lost their homogeneity and are fragmented. The M-band was not clear in sonication samples after freezing.

Conclusion

The evaluation of ultrasound treatments effect on meat tissue post-mortem has shown that sonication influences the character and range of change in protein fraction during rigor mortis. The results may point out that the observed phenomena include changes in enzyme systems activity, both at the beginning of rigor mortis (myofibril structure) and during further storage. As specific tests concerning changes in microstructure have shown, ultrasounds accelerate disintegration of lyzosomes, which then have a stronger influence mainly on the destruction of myofibril proteins. Sonication meat after slaughter shows relatively little effect on the destruction of collagen proteins.

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