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EFFECTS OF HIGH FREQUENCY ULTRASOUND ON AGEING KINETICS, ULTRASTRUCTURE AND SOME PHYSICO-CHEMICAL PROPERTIES OF BEEF MEAT

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OBJECTIVES

Application of high intensity ultrasound in meat and meat products has, for many years, been proposed to increase salt diffusion in meat injected with brine, to prepare fat/water emulsions and to accelerate meat ageing (Sajas and Gorbatow, 1978). It has been shown, on muscle fibers dispersed in a buffer, that low frequency ultrasound can damage cell membranes, release lysosomal enzymes to sarcoplasm and accelerate proteolysis (Roncales *et al.*, 1992). However, when applied on entire meat, high intensity ultrasound at low frequencies (20-40 kHz) failed to significantly improve tenderness in a reproducible way (Smith *et al.*, 1991 ; Lyng *et al.*, 1994).
The aim of the present study was to determine if high intensity ultrasound could be more efficient when applied in a higher frequency range for which the wavelength (<1 mm) corresponds to the size of the primary muscle bundle structure.

METHODS

The *Semimembranosus* muscle of four cull cows were excised one hour after slaughter. Cylindrical samples (H=3cm, ∅=4.5cm) were cut parallel to the myofibres, put in plastic cylinders, vacuum-packaged (film thickness=60µm), kept at about 10°C till *rigor* onset and then at 4°C till measurements.
Ultrasonic treatments were performed in a 6 °C water-bath, so that the sample temperature could not exceed 25°C. The following conditions were used : frequency of 2.6 MHz, 10 cm^2 transducer, power of 10 W/cm^2, duration of 2x15 seconds (15 seconds on each side of sample, with a rest period of 2 min between the two applications). The treatments were performed either in the *pre-rigor* stage (pH 6.3 to 6.1) or in the *post-rigor* period (pH 5.4). Untreated slices were used as controls.
The following determinations have been performed : kinetics of rigor onset at d0 ; delocalization of calcium at d0 on pyroantimonate-osmium fixed samples (Vignon *et al.*, 1989) ; ultrastructural (TEM) modifications at d0, d1, d2, d6 and d14 on samples fixed in 2.5 % glutaraldehyde, 0.1M cacodylate buffer ; release of a lysosomal enzyme, β-glucuronidase, at d2 (Moeller *et al.*, 1976) ; SDS-PAGE at d2, d6 and d14 (Greaser *et al.*, 1986); sarcomere lengths at d6 (Cross *et al.*, 1980); ageing kinetics at d1, d2, d6 and d14 (Lepetit *et al.*, 1986).

RESULTS AND DISCUSSION

Kinetics of *rigor* onset (pH fall). Controls entered *rigor* in approximately 17 hours. The *pre-rigor* ultrasonic treatment induced an immediate increase in pH of about 0.3 unit, which was maintained during several hours and probably slightly delayed the *rigor* onset (Fig. 1). This increase in pH might be due to a release of ions from cell structures into the cytosol. The ultimate pH (~5.5) was, however, similar to that of the controls.

Kinetics of ageing. Mechanical resistance of raw myofibres used as an ageing index is shown in table 1. Ageing is accelerated only in *post-rigor* treated samples (significant difference at d6). However, at d14 no difference in the mechanical indices could be noted.

Release of β-glucuronidase. The β-glucuronidase activities in the cytosolic fraction reflects the release of the enzyme in the cytosol and consequently the fragmentation of the lysosomal membranes. β-glucuronidase activities were expressed as the ratios between sample activity and control activity either in the 30000 x g and in the 100000 x g supernatants (Tab. 1). Ultrasonic treatments have a tendency, more especially when applied at d0, to increase the amount of β-glucuronidase in the two supernatants. However, due to the negative response of one animal out of four, this effect was not significant.

SDS-PAGE of extracted myofibrillar proteins. The 30 kDa band, a marker for myofibrillar degradation, slightly appeared at d6 and then increased in intensity at d14 in controls. The ultrasound applied *pre-rigor* accelerated the myofibrillar degradation as the 30 kDa band was already evident at d2, then increased at d6 and d14. The intensity of the band was higher than that of the control on aged meat (d14). Ultrasound applied after *rigor mortis* also accelerated the myofibrillar degradation, but to a lesser extent : the 30 kDa band was absent at d2, but was more intense at d6 than that of the control; finally on aged meat, no difference in intensity was observed with the control.

Sarcomere lengths. Sarcomere lengths are shown in table 1. Controls exhibited normal sarcomere lengths (2.02 ± 0.14 µm). *Post-rigor* ultrasound treatments lead to significant larger sarcomere lengths (2.09 ± 0.15 µm) ; this increase in sarcomere length is even more marked in *pre-rigor* treated samples (2.22 ± 0.15 µm).

Effect of ultrasound treatment on free cytosolic calcium level. In normal *post-mortem* conditions, the intracellular calcium delocalizes around pH 6.0. This can be evidenced by an increase in intermyofibrillar calcium precipitates during *rigor* onset. The ultrasound treatment has been tested for its ability to accelerate these intracellular changes. The calcium precipitated by the fixation procedure was semi-quantitated in the intermyofibrillar spaces. When measured just after the treatment, the calcium level significantly increased in the intermyofibrillar spaces. Two hours after the treatment, the pH of the controls were at value close to 6.0 and the calcium levels between controls and treated samples were no longer significantly different, although the pH value of the treated samples were still higher (Fig. 2). This suggests that ultrasound is able to induce a large release of calcium in the cytosol early *post-mortem*.

Ultrastructural modifications. No modification was observed just after the *pre-rigor* treatment (d0), except a slight shortening of the sarcomeres which was still observed at d1. At d2, the sarcomeres seemed to be stretched. At d6 (Fig. 3), the sarcomere lengths were large (up to 2.3 µm) compared to control values (around 2.0 µm) which is in agreement with the results of sarcomere lengths measured by laser diffraction. The Z-lines showed alterations at d1 in treated samples. These alterations did not increase with ageing.

The ultrasound treatment at d1 did not induce relevant changes in the ultrastructure.

Table 1 : Results of myofibrillar resistance, β-glucuronidase activities, sarcomere lengths.

Samples*	Myofibrillar resistance** (N/cm²)				β-glucuronidase activities***		Sarcomere lengths
	d 1	d 2	d 6	d 14	S 30000	S100000	(µm)
Control	20.01±6.50 [a]	15.47±7.99 [a]	9.50±3.22 [a]	6.91±2.52 [a]	1.00±0.00 [a]	1.00±0.00 [a]	2.02±0.14 [c]
US 0	22.18±5.00 [a]	16.44±7.28 [a]	9.44±3.83 [a]	7.75±2.34 [a]	1.12±0.27 [a]	1.11±0.31 [a]	2.22±0.15 [a]
US 1	20.37±5.42 [a]	14.87±6.39 [a]	7.21±1.90 [b]	7.89±2.79 [a]	1.09±0.09 [a]	1.05±0.19 [a]	2.09±0.15 [b]

* Samples : Control ; US 0 : ultrasound at pH = 6.2 ; US 1 : ultrasound at pH = 5.4.
** Myofibrillar resistance (N/cm²) measured by a compression test on raw meat under a 20% strain.
*** Ratios between sample and control activities in the 30000 x g (S 30000) or 100000 x g (S 100000) supernatants.
Data in the same column with the same superscript (a, b, c) do not differ (p>0.05).

CONCLUSION

In comparison with low-frequency ultrasonic treatments (20-40 kHz), high intensity ultrasound used at higher frequencies (2.6 MHz) had a much more marked effect on different characteristics of muscle tissues with respect to tenderness and ageing of meat. The release of a lysosomal enzyme (β-glucuronidase), acceleration of the 30 kDa appearance and ultrastructural modifications suggested by the elongated sarcomeres and Z-lines alterations have been observed on high intensity high frequency treatment. The *pre-rigor* application of ultrasound was the most efficient.

However, it is difficult to conclude definitely on the potential of application of high-intensity, high-frequency ultrasound due to the problem of heterogeneity of response of the different animals studied up to now.

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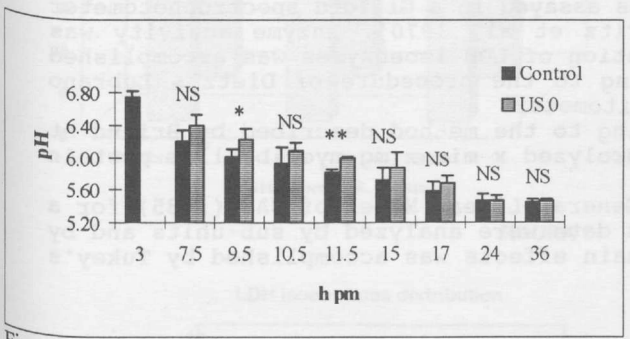

Figure 1. Effects of ultrasound treatment on kinetics of *rigor* onset.
** significant difference (p<0.01); * significant difference (p<0.05); NS: non significant difference (p>0.05).

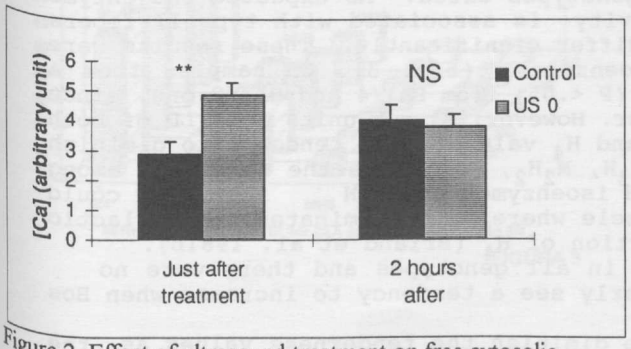

Figure 2. Effect of ultrasound treatment on free cytosolic calcium level (n=3).
** significant difference (p<0.01); NS: non significant difference (p>0.05).

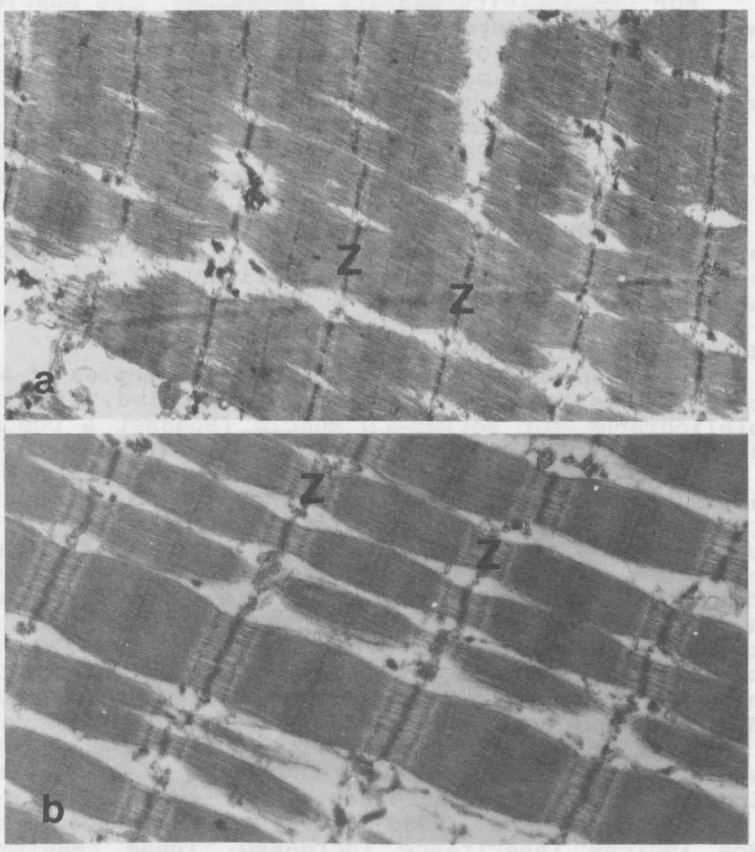

Figure 3. Ultrastructure of control (a) and *pre-rigor* treated sample (b) at day 6 (x 16000) ; Z : Z-lines.