Second harmonic generation microscopy of pse-like zones from pork semimembranosus

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INTRODUCTION

Histological investigations of the PSE-like zones are not frequent in the bibliography, most of the studies are focused on the influence of the main risk factors of this ham's defect. The aim of this work is to test the feasability of a SHG (Second Harmonic Generation) microscopy study of PSE-like zones. SHG microscopy is a rapid tool (fast sample preparation) that allows the three-dimensional study of skeletal muscle. In the present work, this technique has been used to explore the sarcomeric organization of pork Semimembranosus. It may be helpfull to develop a routine protocol for an objective characterization of hams with PSE-like zones, wich are still evaluated with a subjective scale.

MATERIALS AND METHODS

SHG Imaging:

- 5 samples of Semimembranosus muscle were selected at 24 hours post mortem: 2 samples of PSE-zones + 3 control samples
- muscles were fixed for 36 hours in 4% paraformaldehyd and sectioned for SHG imaging (100µm to 1mm sections)
- imaging system: Leica TCS SP2 confocal scanning head coupled to a DMIRB invertedmicroscope and equipped with a MAITAI Spectra Physics femtosecond laser.

The frequency of sarcomeric single band signal was estimated with random field determination (100 fields per samples ; 10 fields separated with 4µm minimum distance and repeated 10 times in the sample thickness). Six axes were used within each image to determine the single band/double band ratio.

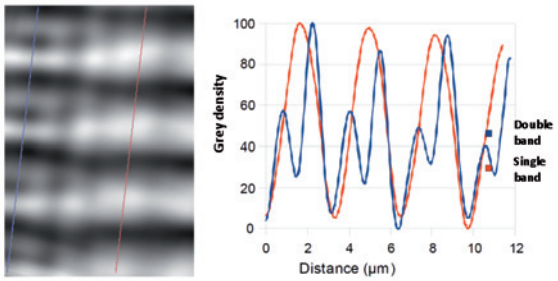

Fig1. axis determination of single/double band ratios using the distance between grey density peaks in SHG image sample

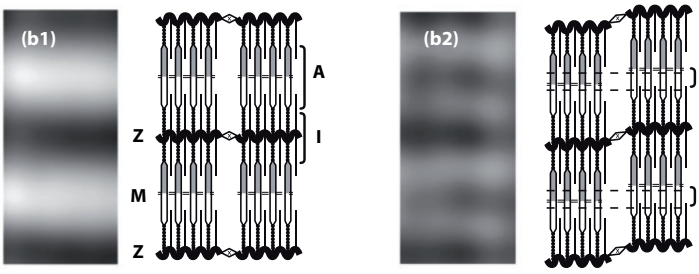

Fig2. sarcomeric organization and SHG imaging. (b1) = aligned sarcomere (in vivo) and single band SHG signal; (b2) = non aligned sarcomere (proteolysis) and double band SHG signal

Electron Microscopy:

- Extra samples of PSE-zones and control Semimembranosus were treated for the needs of electron microscopy (80 kev JEOL 100CXII)

RESULTS

- The single band/double band ratio differs for PSE-zones and control samples (35% vs 87.5% of single band, respectively, figure 3)
- SHG imaging shows less proteolysis in PSE-zones despite the high level of proteolysis observed in both control and PSE-zones by electron microscopy (figure 4)
- Breaks in the myofibril lenght observed in PSE-zones could help to maintain myofibrill alignment

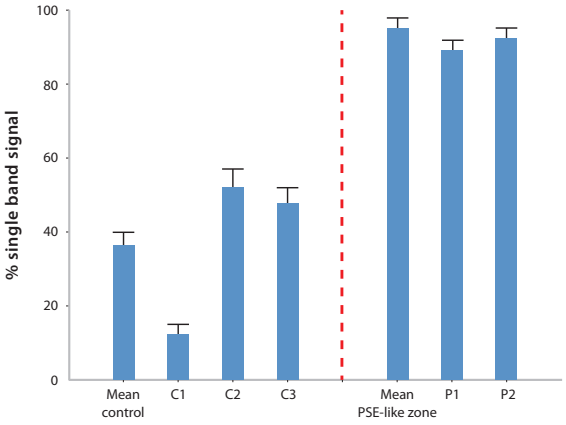

Fig3. frequency of SHG single band signal for sample with PSE-like zone and control sample (mean, SEM)

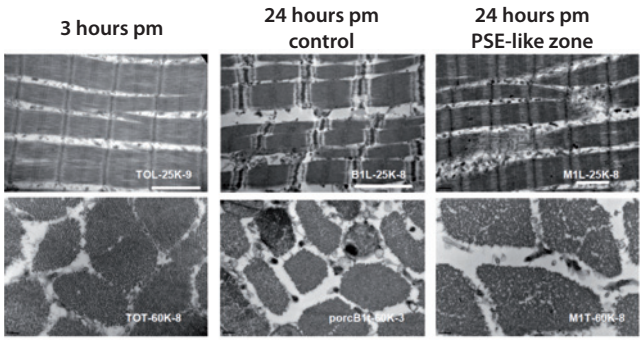

Fig4. electron microscopy images of Semimembranosus at 3h and 24h post mortem (control and with PSE-like zone)

Conclusions

The results of this experiment indicate that SHG pattern may be a relevant technique for exploring the meat structure. In the PSE-like zone defect issue, single/double band ratio seems to be an objective indicator of the structure problems of muscle. It could be a more precise criterion than the IFIP subjective scale. SHG microscopy is a label-free and lot more rapid technique than classical histology and may be carried out routinely for experiments focusing on PSE-like zones or other kind of meat structure defect.