SECOND HARMONIC GENERATION MICROSCOPY OF PSE-LIKE ZONES FROM PORK SEMIMEMBRANOSUS

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Abstract – The aim of this work was to study the sarcomeric organization of muscles with PSE-like zones and normal muscle (control), focusing on the SHG signal pattern of myosin (single or double band). Second harmonic generation microscopy has been applied on pork *Semimembranosus* samples fixed at 24 hours post mortem. Electronic microscopy was applied on the same samples to improve SHG images interpretation. PSE-like zones were presenting SHG signal with a higher single band frequency than the control muscles (87.5% vs 35%, respectively). The defect SHG pattern was close to the data obtained on skeletal muscle in normal physiological conditions. This result indicates that the sarcomeric alignment is mostly maintained in PSE-like zone whereas the alignment is over in normal muscle. Electronic microscopy images confirmed this data. This study reveals that SHG microscopy could be a new tool to identify structure defects in pork muscles at 24h postmortem, in addition to the subjective PSE-like zone scale.

Key Words – Pork meat, Structure defect, SHG microscopy

I. INTRODUCTION

PSE-like zone meat is still a big issue for the cooked ham industry. From a 4 to 17% basal frequency observed on pork raw hams for classical experimental conditions [1, 2, 3], the defect rate had risen to 44% under altered slaughter conditions (reduced fasting time and no resting before slaughter) and with a high rate of heterozygous halothane genotype [4]. Despite studies concerning some of the main risk factors (fasting time, transport and resting time, chilling rate, halothane sensitivity genotype) histological investigations of the PSE-like zone are not frequently found in bibliography. The defect is characterized by a disorganization of myofiber alignment, disrupted fibers with an increased extracellular space [2, 5]. The present study is focusing on the opportunity to explore myofibrillar structure of PSE-like zones with second harmonic generation microscopy. Such a technique has been recently used on Xenopus to study the muscular structure based on the endogenous myosin protein [6]. SHG microscopy is a rapid tool (simple sample preparation) that allows three-dimensional study of skeletal muscle. This study was achieved with two main objectives, to explore sarcomeric organization of pork S*emimembranosus* with PSE-like zone, and to develop a routine protocol for an objective characterization of this defect by SHG imaging.

II. MATERIALS AND METHODS

Five samples of *Semimembranosus* muscle were selected at 24 hours post mortem from deboned hams of Pietrain sire crossbreed. The 2x2x5cm samples were presenting or not PSE-like zones, according to the IFIP scale quotation (class 1 or class 4 - [7]). Samples were immediately placed in PBS (Phosphate Buffer Sodium) containing Ca^{2+} (1mM) and Mg^{2+}(2mM) during transport to the laboratory, then fixed for 36 hours in 4% paraformaldehyd. Large sections of *Semimembranosus* (100µm to 1mm) were placed in a 50% Glycerol solution for Second Harmonic Generation (SHG) image analysis (Olympus FV1000 MPE microscope equipped with a 940nm impulse laser source Matai HP Spectra-Physics fentoseconde). Image acquisition and analysis were performed with the Olympus Fluoview 2.1 software. The frequency of sarcomeric single band signal [8] was estimated with a random field determination (512x512 pixels) based on a total of 100 fields per sample (10 fields separated with a 4µm minimum distance and repeated 10 times in the sample thickness). Six axes were used within each image (pixel 0, 100, 200, 300, 400 and 500) to determine the single/double band ratio.

Extra samples of *Semimembranosus* ($1mm^3$) from PSE-like zones or control were examined with an

electronic microscope in order to describe the sarcomeric organization. Paraformaldehyd fixed samples were placed for 4 hours in a 2.5% glutaraldehyd solution at 4°c, then washed in a 0.2M cacodylate buffer overnight. After being treated with 2% osmium and rinsed with cacodylate buffer, samples were dehydrated successively with 70% to 100% acetone solution. Epon, Araldite®, and acetone were added to the samples for 90 minutes, then Epon, Araldite® and DMP30 for the same time. Sample polymerization was achieved overnight at 60°c. After a 24 hours dehydration, ultrathin slices were obtained with a diamond type slicer, then treated with 2.5% uranyle acetate for an hour and with citrate lead for 20 minutes. Samples were examined with a 80 kev electronic microscope JEOL 100CXII.

III. RESULTS AND DISCUSSION

Both sarcomeric single band and double band SHG signal were found on *Semimembranosus* samples at 24 hours post mortem (figure 1). When sarcomere alignment is maintained, as observed for adult muscles in normal physiological conditions, the SHG signal pattern reveals predominantly the single band form [6]. Double band signal occurs when sarcomeres are not aligned. It happens on fresh muscle placed in vitro under oxidative conditions (H2O2, [9]). Double band can be observed too during the post mortem tenderization process of meat due to the proteolytic action of caspases and calpaïns on structure proteins like titine and desmine (figure 2). For this reason, the frequency of double band signal can be used to estimate the proteolysis level of the muscle.

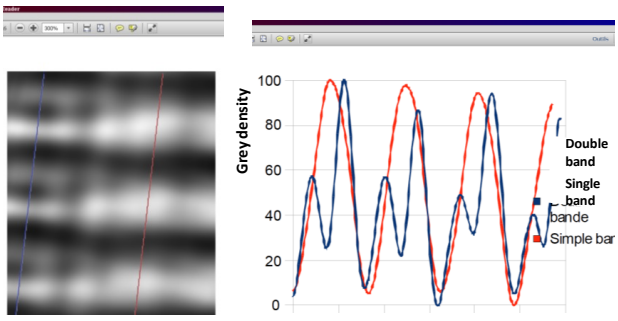

Figure 1. Axis determination of single/double band ratios using the distance between grey density peaks in SHG image sample

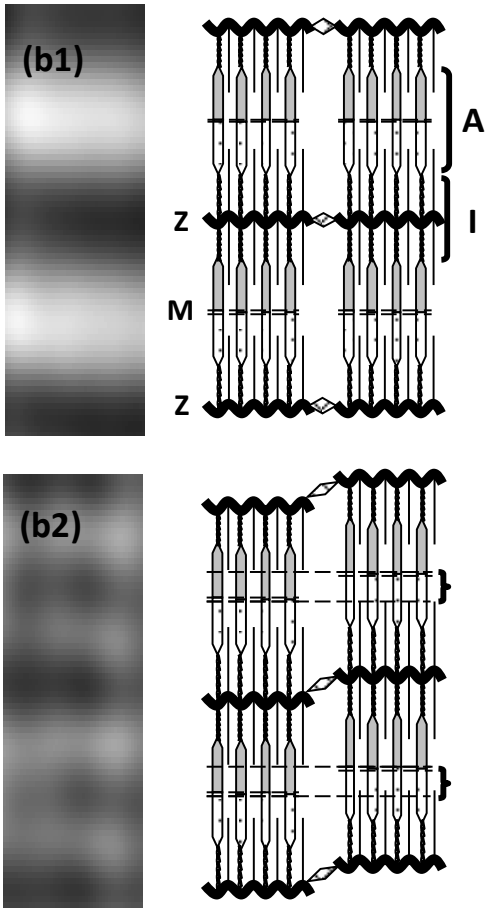

Figure 2. Sarcomeric organization and SHG imaging. (b1) = aligned sarcomere (in vivo) and single band SHG signal; (b2) = non aligned sarcomere (proteolysis) and double band SHG signal

Control samples of *Semimembranosus* and samples with PSE-like zone showed a strong single/double band ratio difference, 35% and 87,5% of single band, respectively (figure 3). SHG imaging results for PSE-like zone are close to results obtained on early post mortem or in vivo muscle. These data are in good agreement with 2-D electrophoresis results already available [5] showing less proteolysis in PSE zones.

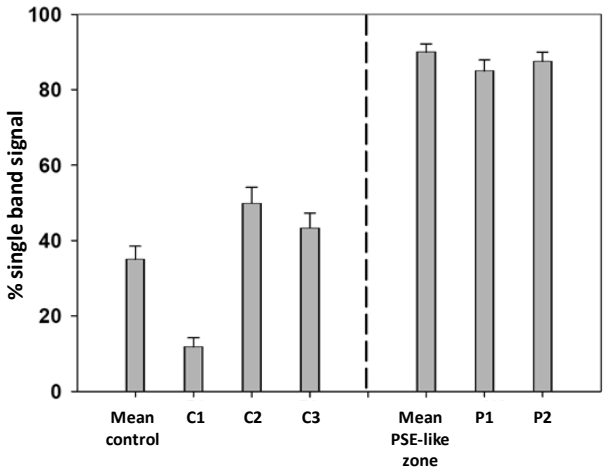

Figure 3. Frequency of SHG single band signal for sample with PSE-like zone and control sample (mean, SEM)

Electron microscopy imaging is in agreement with SHG imaging results. The figure 4 indicates clearly a high level of proteolysis at 24 hours in both control and PSE-like zone samples. Nonetheless, the non-alignment of sarcomeres induced by post mortem proteolysis in control samples is not observed at 24 hours post mortem on samples with PSE-like zone, where sarcomere alignment is maintained. Breaks in myofibril length that were previously noticed on PSE-like zones [5] clearly appear in figure 4. These breaks may induce a reduction of mechanical tension in the myofibril length and between myofibril. As a consequence, a myofibrillar relaxing may happen and could help to maintain sarcomere alignment.

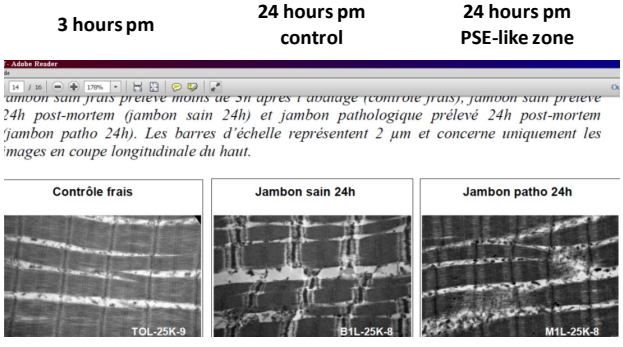

Figure 4. electron microscopy images of *Semimembranosus* at 3h and 24h post mortem (control and with PSE-like zone)

IV. CONCLUSION

The results of this experiment focusing on 24 hours post mortem pork muscles histology 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 grade. 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.

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