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FAST METHODS FOR DETECTING MUSCLE PROTEIN DENATURATION DUE TO SALTING

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I. INTRODUCTION

Meat preparations and meat products are defined in the 853/2004 EC regulation with a major difference that consist in muscle structure modifications and fresh meat characteristics loss, in the case of meat products. In contrast, meat preparation still shows macroscopic and microscopic muscle structure. The addition of salt, used in meat processing as a flavour enhancer, preservative, and texture-control agent, lead to protein denaturation. Salt effect on isolated myofibers is known to produce Z-lines degradation, swelling and/or disappearance of sarcomere anisotropic regions observed by electron microscopy [1]. These changes are due to solubilization of myofibrillar proteins in the presence of salt [2]. On sausage meat, a salt amount higher than 1.3% could result in considering the sausage as a meat-based product [3]. The aim of this experiment was to test different technologies for grading meat according to their salt content, to help classification of product according to 853/2004 EC regulation for high salt content meat, like “petit salé”. Visible and Near Infrared (VisNIR) spectroscopy due to the water peaks in the spectrum, and Second Harmonic Generation (SHG) imaging for its very simple sample preparation [4], were studied as histology alternatives.

II. MATERIALS AND METHODS

24 pork carcasses from IFIP experimental farm were selected at slaughterhouse during 3 trials, and both right and left *teres major* muscles were sampled after deboning of shoulders. The *teres major* muscle was chosen for its high homogeneity, low intramuscular fat and connective tissue content. 7 treatments were applied before the 16h tumbling process according to the brine composition (10% injection): physiological fluid only, brine added with 1.1%, 1.3%, 1.5%, 1.7% or 1.9% of sodium chloride. *Teres major* cross sections were measured with the VisNIR spectrometer ASDI Labspec4 and PLSDA classification models were built by random cross validation, using Matlab 7.10.0 software (Natick, USA) and Eigenvector toolbox (Manson, USA) after baseline correction, smoothing and GLS weighting spectra treatment. A subsample of 3 carcasses was selected and *teres majo*r samples were collected and fixed with 4% paraformaldehyde phosphate buffer prior to SHG image analysis (Olympus FV1000 microscope equipped with a 940 nm impulse laser source). Image acquisition and analysis were performed with the Olympus Fluoview 2.1 software. The frequency of sarcomeric single band and double band signal was estimated with a random field determination based on a total of 100 fields per sample

III. RESULTS AND DISCUSSION

After the tumbling process, teres major muscles with 1.1% to 1.9% of salt increased their water content of 6% (no significant difference, results not shown) while the physiological fluid treatment showed a 2.5% water content increase ($p<0.05$). In SHG Imaging, double band signal occurs when sarcomeres are not aligned. It can be observed during the *post mortem* tenderization process of meat due to the proteolytic action of caspases and calpaïns on structure proteins like titine and desmine [5]. The percentage of double band signal increased from 1.3% NaCl group (73%) to 1.9% NaCl group (99%), confirming proteolysis effect of salt (table 1). On the other hand, the overall sarcomere SHG signal rate (single band + double band) was found to be more discriminant with a strong signal decrease from 1.3% NaCl (83%) to 1.5% NaCl (26%), 1.7% NaCl (15%) and 1.9% NaCl (4%). These preliminary results on 3 muscles samples revealed the potential of SHG imaging to discriminate the 1.5% salt content threshold. From this point, only a minority of analysed fields showed organized sarcomeres (figure 1).

The PLSDA models based on VisNIR spectra showed very low error level in cross validation for every salt content threshold (0%, 0.7%, 0%, 1% and 0.3% of misclassification for 0-1.1%, 1.1-1.3%, 1.3-1.5%, 1.5-1.7% and 1.7-1.9% groups, respectively – table 2). The discrimination of the n=305 spectra data base with a moving salt content threshold seems to be characterized with a very stable error despite reduced R^2cv for the 1.5-1.7% and 1.7-1.9% salt content threshold ($R^2cv=0.27$ and 0.42, respectively). However, the unbalanced population size for the extreme threshold values will require larger sampling and external validation to confirm the classification error.

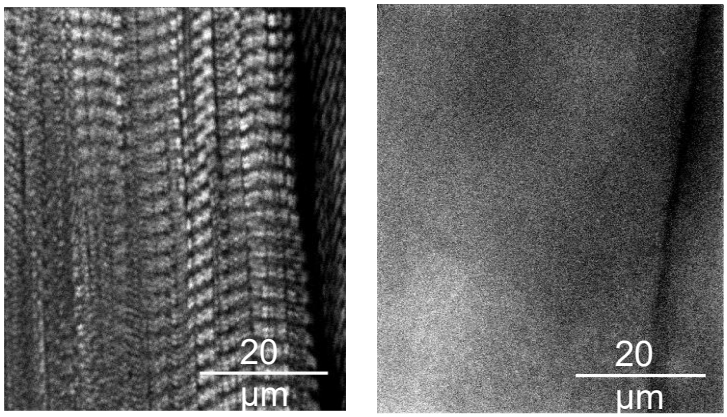


Figure 1. SHG imaging fields of *teres major* after salting (double band signal up to 1.3% salt, and no organized sarcomere signal from 1.5% to 1.9% salt, left and right respectively)

Table 1. SHG imaging results

NaCl (%) group	Double/single band SHG signal ratio (%)	Field ratio with SHG signal (%)
0	85	73
1.1	82	43
1.3	73	83
1.5	99	26
1.7	99	15
1.9	99	4

Table 2. Cross validation results for PLSDA classification of salt content groups with VisNIRS

Nacl (%) threshold	Spectra pre-treatment	Nb PLSDA factors	R^2_c	R^2_{cv}	Cross validation misclassification
[0] – [>1.1]	Baseline correction + smoothing + GLS weighting	2	0.92	0.85	0/305
[< 1.1] – [> 1.3]		3	0.88	0.62	2/305
[< 1.3] – [> 1.5]		4	0.86	0.54	0/305
[< 1.5] – [> 1.7]		5	0.73	0.27	3/305
[< 1.7] – [> 1.9]		2	0.69	0.42	1/305

IV. CONCLUSION

This preliminary study revealed the potential of VisNIR spectroscopy and SHG Imaging to distinguish salt content of muscle after a tumbling process. Considering that NaCl is known to lead to the disorganization of sarcomeres at proper concentration, SHG imaging may become a new standard for defining microscopic organization state of salted muscles according to the EC regulation 853/2004 criteria. VisNIR spectroscopy showed very promising results for classification of meat according to its NaCl content whatever the threshold value, but needs a larger experiment with external validation samples to get confirmed.

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