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Deciphering PSE-like muscle defect in cooked hams: A signature from the tissue to the molecular scale

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ABSTRACT

PSE-like technological defect in the meat industry is of great importance due, to the economic loss it can cause. It has been studied from the biochemical perspective but very few studies have focused on tissular characterization. This study proposes innovative approaches that combine mechanistic elucidation and the discovery of potential biomarkers. This study focused on muscle destructuration using imaging and label-free quantitation. Oxidative stress and apoptotic processes were found to be linked to the specific evolution of the PSE-like destructuration zone, namely 'inner', within hams. Four *m/z* values were found to be related to the specific localization of the PSE-like defect, and a mass shift of 27 Da suggested a possible connection with oxidation. These potential markers of the PSE-like area in ham provide a new perspective to sort raw material based on the possible development of PSE-like areas.

1. Introduction

The PSE-like muscle defect occurring in cooked hams is a major issue in French "jambon supérieur" cooked ham, which is polyphosphate-free. Its occurrence can affect up to 50% of the total processed hams, and leads to significant economic losses (Vautier, Boulard, Houix, & Minvielle, 2008). Indeed, it is only when the automated slicing process takes place that the PSE-like muscle defect and holes within the ham are revealed. Due to histological and biochemical similarities, this defect is comparable to PSE-meat, but in most cases it is located in the deep regions of the *semimembranosus* muscle (Vautier, Boulard, Houix, & Minvielle, 2008).

The PSE-like defect has been widely investigated. It was found to be highly correlated with a low ultimate pH value (Franck et al., 1999; Hugenschmidt et al., 2010; Le Roy et al., 2001), and highly correlated with a high slaughter weight (Franck et al., 1999). The PSE-like defect was also studied on the basis of the genetic component, and it appeared that the presence of the n allele of the Hal-gene, and that of the RN^- allele of the RN-gene intensified the defect (Franck, Figwer, & Poirel, 2000; Le Roy et al., 2001). A biochemical study showed that the PSE-like defect was strongly correlated with glycolytic potential (Minvielle, Le Strat, Lebret, Houix, Boulard, & Clochefert, 2001), which was confirmed by Laville et al. (Laville et al., 2005) who conducted a proteomic study on destructured raw meat. They found out that the defect resulted from abnormally fast *post mortem* glycolysis.

Although previous observations suggested an internal gradient leading to a heterogeneity of the defect (Franck et al., 1999), no information is available regarding the progression of the PSE-like area within hams. Moreover, a recent study investigated the use of near-infrared spectroscopy to classify fresh pork meat and predict the suitability of fresh pork for the production of cooked ham (Neyrinck et al., 2015). In this context, the aim of this study is to elucidate the development of the PSE-like defect, by characterizing it from the tissue to the molecular scale and determining its localization within the *semimembranosus* muscle. This muscle was chosen because it was previously described as one of those most affected by the defect (Laville et al., 2005) and because of its localization in the inner part of the ham. Therefore, the experiment was designed to answer two questions: does the PSE-like defect progress internally within the ham, with links to the muscle's internal structure; and can the PSE-like defect be observed from the outer part of the ham? In other words, is there a biological marker available on the outer part of the boneless ham?

A multimodal approach was applied: first, a proteomic approach was used, combining label-free protein quantitation and identification

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by mass spectrometry, to determine which biological processes were involved in destructuration of the hams. Mass spectrometry imaging was applied to determine the spatial variability of molecular signature related to hams destructuration within the *semi membranosus* muscle. Imaging by matrix-assisted laser dissociation ionization – time-of-flight (MALDI-TOF) mass spectrometry can be used to analyze the spatial distribution of a wide variety of molecules, such as lipids, metabolites, peptides, and proteins, simultaneously within a single tissue section (Caprioli, Farmer, & Gile, 1997). This method is widely used to study biological phenomena because of its capability to map hundreds of molecules in one mass spectra acquisition sequence without any labeling. So it can be applied to any species, unlike histo-immunological approaches. The use of 24 h *post mortem* samples to define PSE-like defect biomarkers was justified by pH and temperature stabilization. Indeed, it facilitates the measurements and make them more reliable. Moreover, the novel application of this multimodal approach will help to better understand the mechanisms underlying the occurrence of the PSE-like defect.

2. Material and methods

2.1. Experimental design

A batch of 120 pigs (from a sow Large White × Landrace and Pietrain sire), from different farms, were slaughtered at the commercial abattoir "la Guerche de Bretagne, France". At 24 h *post mortem*, after pH and temperature stabilization, hams were sorted according to their level of destructuration on a scale from 1 to 4 (Vautier, Boulard, Houix, & Minvielle, 2008) (Fig. 1A), their pH, temperature, and exudate values (Supplementary data 1). From the 120 animals, 20 were selected, based on the destructuration score: 10 'normal hams' (NH) and 10 'PSE-like hams' (DH). The inner part (DHi) and outer parts (DHo) of *semimembranosus* muscles were sampled in each of the 'PSE-like hams'; and the inner part of *semimembranosus* muscles was sampled in each of the 'normal hams' (NH) (Fig. 1B).

These 30 samples were part of this multimodal approach:

- proteomic analysis with label-free quantitation of proteins by LC-MS/MS,
- imaging, combining tissue structure characterization by histology and mass spectrometry imaging (MSI) by MALDI-TOF.

2.2. Tissue preparation

At 24 h post mortem, *semimembranosus* muscle samples (1 × 1 × 1 cm) were collected, positioned on a cork plate and cryofixed by immersion at −160 °C in isopentane cooled with liquid nitrogen (−196 °C). Serial cross-sections (10 µm thick) were cut using a cryostat (Microm HM 560; Thermo Scientific). They were collected on glass slides for histological stains and on indium tin oxide (ITO) glass slides (Bruker Daltonik, Bremen, Germany) for MALDI-MSI (Fig. 1C). The glass slides were stored under vacuum until use.

2.3. Histology

Sections were stained with Hematoxylin–Eosin (HE) to contrast the muscle cells and Sirius Red to contrast collagen (Astruc, Labas, Vendeuvre, Martin, & Taylor, 2008). Observations and image acquisitions were performed using a light microscope (Olympus BX 61) coupled to a high-resolution digital camera (Olympus DP 71) with CellSens software.

2.4. MALDI-TOF mass spectrometry imaging

2.4.1. Acquisition

The MALDI-TOF-MSI experiments were performed on muscle sections collected on conductive indium-tin-oxide glass slides (Bruker Daltonics, Bremen, Germany). The muscle sections were subjected to two washing steps at 70% and 95% ethanol to deplete lipids, and then dried in a desiccator for 30 min. The sinapinic acid matrix was 10 mg/mL in water/acetonitrile at 60:40 (*v/v*) with 0.2% trifluoroacetic acid. The sinapinic acid was then applied using ImagePrep (Bruker Daltonics), according to the manufacturer's recommendations. All the ImagePrep phase settings for matrix deposition are detailed in Supplementary Data 2.

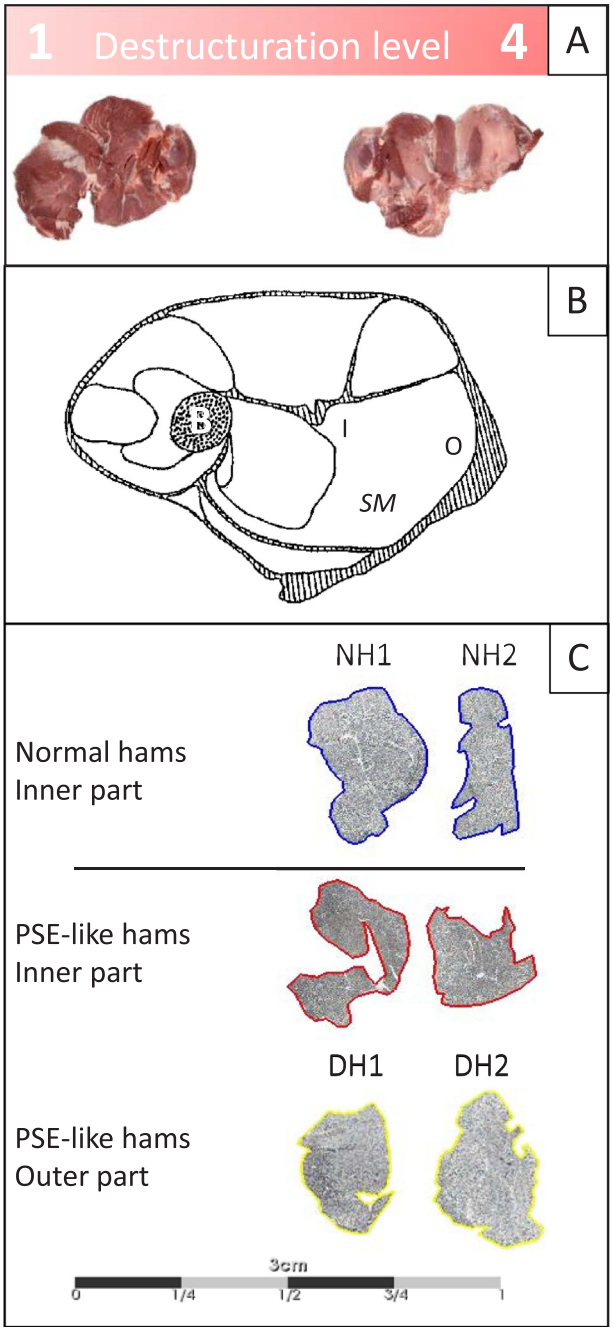

Fig. 1. Experimental design. A: Classification according to the level of muscle destructuration, on a scale from 1 to 4 (Vautier, Boulard, Houix, & Minvielle, 2008). B: Sampling points of the muscle cross-section in their anatomical position: inner (I) and outer (O) part of the *semimembranosus* (SM) muscle, and the bone (B) (from Sayd, Mera, Martin, & Laville, 1998). C: Examples of cross-sections from 2 of the 10 'normal hams' samples (NH), 2 from the 10 samples from the inner part of the 'PSE-like hams' (DHi), and 2 from the 10 samples from the outer part of the 'PSE-like hams' (DHo).