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Application to proteomics to understand and modify meat quality

M. Gobert, T. Sayd, P. Gatellier, V. Santé-Lhoutellier *

INRA QuaPA, F 63122 saint Genès Champanelle, France

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ABSTRACT

The use of proteomics in the field of meat science has gained in robustness and accuracy. This is consistent with the genomic and bioinformatic tools. Its application to sensorial and technological meat quality traits is discussed as well as the emergence of sanitary and nutritional issue which will grow in a next future.

1. Introduction

Meat quality is a broader expression in the meat science which covers different aspects considering the targets. For the consumer, it is clear that a safe product is a prerequisite. Besides that, the qualities searched by consumer are related to their physiological needs and hedonic to fulfill dietary needs but also those the competitiveness of the industry. In the 2000s, several reviews dealing with proteomics focused on technical developments about proteomics, post genomics tools and their limits (Bendixen, 2005; Mullen, Stapleton, Corcoran, Hamill, & White, 2006), enhancing product quality (Hollung, Veiseth, Jia, Faergestad, & Hildrum, 2007;) or the species specificity (Paredi, Raboni, Bendixen, de Almeida, & Mozzarelli, 2012; Picard et al., 2010; Van de Wiel & Zhang, 2007). In 2013, the review of Paredi et al. (2013) has taken the side to put the use proteomics in meat science in a farm to fork perspective including processing and safety, while the review of Bendixen, Danielsen, Hollung, Gianazza, and Miller (2011) focused on how proteomics would allow to get a better and more efficient production of farm animals. This list is not intended to be fully exhaustive but more for illustrative purposes of the importance of the field of proteomics and its application.

The explosion of publications in the two last decades has profited from the improvements on the reproducibility and robustness of the methods and the analytical tools. A breakthrough occurred with the sequencing of the genome of livestock. In 2004, just three years after the sequence of human genome, the first version of the sequence of the chicken genome was published (Hillier, Miller, Birney, et al., 2004). This work is the result of over ten years of research in genomics. In 2009, the genome of a female Hereford cow has been sequenced by the Bovine Genome Sequencing and Analysis Consortium, a team of researchers led by the National Institute of Health and the U.S. Department of Agriculture (Elsik et al., 2009). It is one of the largest genomes ever sequenced. In 2012 the genome of a Duroc pig was sequenced. It joined the growing list of pets and livestock whose genome has been sequenced. These results are likely to have a major impact on livestock breeding and for progress in medicine. The pig serves as a research model for human disease because pigs are very similar to human physiology in their behavior and nutritional needs.

In a cell, the protein corresponds to the "end product" of a gene, mercilessly summarizing. However, regulation of gene expression through epigenomic factors, themselves influenced by environmental factors. Proteomics allows the study of the protein content at a given point in time. This holistic view requires the development of high throughput tools and approaches for monitoring gene expression and protein profiles and for determining protein function and interactions. Up to now, proteomic studies have been employed for relating one or two quality traits to a combination of potential biomarkers, trying to gain more knowledge on the physiology and biology of animal, muscle, cell or molecules. Recently, D'Alessandro and Zolla (2013) provided a comprehensive review of on the application of proteomics to farm animals, and especially the muscle to meat conversion, highlighting the emergence of non-proteomics "omics" such as the study of mitochondrial RNAs (miRNAomics, protein post-translational modification PTMomics, metabolomics, lipidomics, …). Then it is questionable whether to integrate all these data to get the very essence. It must be noted that the mechanisms underpinning the main meat qualities are only partially understood. This review will focus on the recent advances on the classical traits of the meat quality such as those involved in sensorial qualities,

* Corresponding author.
E-mail address: veronique.sante@clermont.inra.fr (V. Santé-Lhoutellier).

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but also emerging traits such as those involved in sanitary and nutritional traits.

2. Sensorial quality application

2.1. Colour

The colour of meat is the result of the haeminic pigment content and the oxidoreduction state of the haem iron, highly influenced by the muscle cell environment (metmyoglobin reduction enzyme activity and oxygen consumption). Therefore the meat included its stabilty during storage. Meat colour defect is often considered by consumers as an indication of spoilage and unwholesomeness, leading to rejection of the meat products. Therefore the meat colour is one of the most important traits to be controlled in the meat chain especially for fresh meat. From an economical point of view, the cost of discolouration induced quality was estimated over 1 billion dollar for the meat industry in US (Smith, Belk, Sofos, Tatum, & Williams, 2000). The meat colour desirable for consumer is firstly species dependent: cherry red for beef, pink for pork and pale pink for poultry, and secondly muscle dependent. The advances in proteomics on this particular trait have shed light on several proteins which contribute to colour stability, mostly chaperones and antioxidant proteins. For example, Sayd et al. (2006) showed that the metabolism of pig muscle leading to darker meat was more oxidative oriented, as it was shown by more abundant mitochondrial enzymes of the respiratory chain, hemoglobin, and chaperone or regulator proteins (HSP27, αB-crystallin, and glucose-regulated protein 58 kDa). Conversely, enzymes of glycolysis and glutathione S-transferase ω were overexpressed in the muscle leading to lighter meat. This is consistent with faster postmortem metabolism, namely, acceleration of ATP depletion and pH fall and subsequent enhanced protein denaturation, well-known to induce discoloration. In beef, the study of Joseph, Suman, Rentfrow, Li, and Beach (2012) showed a higher abundance of peroxiredoxin-2, peptide methionine sulfoxide reductase, and heat shock protein-27 kDa and a positive correlation with colour stability. In other words the color stability of *Longissimus* could be attributed to the overabundance of antioxidant proteins and chaperones. These authors recommended developing muscle-specific processing strategies to improve beef color. The study of Hwang, Park, Kim, Cho, and Lee (2005) concluded in the same way. Most proteins cited having antioxidant properties, highlighting the major role of oxidation processes in the meat colour and its stability during storage. In addition it puts forward the ability of muscle through its endogenous antioxidant enzymes and antioxidant vitamins to resist to oxidation. In the meat, the oxidation is due to the production of reactive oxygen species such as free radicals. This production is catalysed by transition metals such as copper and especially iron in the case of muscle. In live animals, even in the case of oxidative stress such as handling conditions, the cellular mechanisms of detoxification permit to recover an equilibrium. Indeed in response to the production of ROS (superoxide O_2^-, hydrogen peroxide H_2O_2, hydroxyl radical OH^-), the antioxidant system is highly efficient to scavenge or detoxify excess ROS. However within a few hours of animal slaughter, the impact of these free radicals is critical for the muscle cell as its antioxidant protection decreases. The myoglobin oxidation is very linked to lipid oxidation (Baron & Andersen, 2002). Recent developments in proteomics highlighted the role of reactive aldehydes such as HNE (4 hydroxynonenal) or HHE (4 hydroxy-hexenal) to make myoglobin adducts mainly via Mickael addition, in other words through the reactivity of their double bond to amine residues. Suman, Faustman, Stamer, and Liebler (2006, 2007) used mass spectrometry to identify which histidines were involved via its imidazole residue to the adduction of HNE. This work indicated that for beef, histidines 81 and 88 were preferentially adducted. Moreover the ultimate pH of the meat combined with the temperature of storage conditions would decrease the potential of HNE to make Mickael adducts. One explanation would be the protonation of imidazole ring at low pH, leading to a nucleophility reduced. Then the histidines of myoglobin would be a target less favorable to HNE adduction.

2.2. Intramuscular fat

This trait is of interest for sensorial qualities and marbling with reasonable amounts of intra muscular fat is being sought from now. In farm animal lipids are distributed among in different tissues; however for livestock producer, controlling lipid deposition in muscle is of importance to fit the consumer demand (Fernandez, Monin, Talmant, Mourot, & Lebret, 1999). The main difficulty is that intramuscular fat has been rarely studied per se but as a component of the determinism of another quality trait: tenderness (Laville et al., 2007). Recently Fuentes, Ventanas, Morcuende, and Ventanas (2013) demonstrated that temporal perception of hardness increased with the IMF content, highligting the key role of lipid in textural properties of meat products. However specific proteomic studies have been carried out to get a deeper knowledge on the promotion of adipogenesis and IMF accumulation instead of cell metabolism (Katsumata, 2011). In this context, the role of dietary lysine level was shown to regulate glucose transporter protein mRNA expression. In addition higher mRNA abundance of peroxisome proliferator-activated receptor γ, a master regulator of adipogenesis in *Longissimus* was induced by dietary low lysine. More than 80% of IMF is stored in adipocytes interspersed in the perimysium and less than 20% located within cytoplasm of myofibers (Gondret, Guitton, Guillerm-Regost, & Louveau, 2008). Proteomics was conducted to characterize the adipocytes according to their location (intra muscular, intermuscular, sub cutaneous and perirenal adipose tissue). The comparisons revealed that more than one hundred spots were differently expressed between intra muscular adipocytes and the fat cells derived from the 3 other adipose locations. The proteins involved in lipogenesis (cytosolic malate dehydrogenase and isocitrate dehydrogenase), glycolysis (enolases and aldolase), lipolysis (perilipin), and fatty acid oxidation (long-chain fatty-acyl CoA dehydrogenase) were downregulated in intramuscular adipocytes. These results suggested that the metabolic activity of intramuscular adipocytes is lower than in the other fat tissues. They concluded that triggering adipogenesis rather than cell metabolism per se might be a valuable strategy to control lipid deposition in pig skeletal muscles.

2.3. Tenderness

The search for an optimal tenderness generates a broad consensus. A lot of work has been done on the comprehension of the structural and biochemical mechanisms involved in meat tenderness. A remarkable schematic overview of the intricate pathways leading to tenderization was proposed by D'Alessandro and Zolla (2013); however it is difficult, if not impossible to quantify the cascade of hundreds of enzyme reactions, and establishing a hierarchy. The tenderization process starts after animal slaughter by biochemical and physical changes. The first step is the shift from aerobic to anaerobic metabolism to generate energy (ATP Adenosine Triphosphate Dehydrogenase) for maintaining muscle cell homeostasis. However, the APT disappearance causes to the production of protons, i.e. pH decline and the muscle stiffness. Then the second step includes the action of a set of enzymes involved in proteolysis, oxidation, to mention only the main class of enzymes. The comprehensive review of Lomiwes, Farouk, Wiklund, and Young (2014) on the role of chaperone proteins in the determinism of meat tenderness related their implication in the different events taking place from ante mortem to post mortem and post rigor. For example, their function in unstressed cell is fundamental for polymerization of the actin microfilament as well as the regulation between filaments. Under stress, slaughter can be assimilated to a stressful event; their function is more oriented as a molecular chaperone and anti apoptotic. Most of the studies on tenderness prediction revealed the HSPs as potential biomarkers (Kim et al., 2008; Laville et al., 2009; Polati et al., 2012, to cite a few).