Meat Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

Proteomic approach to characterize biochemistry of meat quality defects

M.W. Schilling[a,*], S.P. Suman[b], X. Zhang[a], M.N. Nair[b], M.A. Desai[c], K. Cai[a], M.A. Ciaramella[d], P.J. Allen[e]

[a] Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS 39762, United States
[b] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, United States
[c] Reed Food Technology, Pearl, MS 39208, United States
[d] New York Sea Grant, College of Agriculture and Life Sciences, Cornell University, Stony Brook, NY 11794, United States
[e] Department of Wildlife, Fisheries, and Aquaculture, Mississippi State University, Mississippi State, MS 39762, United States

ARTICLE INFO

Keywords:
Proteomics
Meat quality
Woody breast
PSE (pale, soft and exudative) meat
Red-catfish fillet
Myoglobin oxidation

ABSTRACT

Proteomics can be used to characterize quality defects including pale, soft, and exudative (PSE) meat (pork and poultry), woody broiler breast meat, reddish catfish fillets, meat toughness, and beef myoglobin oxidation. PSE broiler meat was characterized by 15 proteins that differed in abundance in comparison to normal broiler breast meat, and eight proteins were differentially expressed in woody breast meat in comparison to normal breast meat. Hemoglobin was the only protein that was differentially expressed between red and normal catfish fillets. However, inducing low oxygen and/or heat stress conditions to catfish fillets did not lead to the production of red fillets. Proteomic data provided information pertaining to the protein differences that exist in meat quality defects. However, these data need to be evaluated in conjunction with information pertaining to genetics, nutrition, environment of the live animal, muscle to meat conversion, meat quality analyses and sensory attributes to understand causality, protein biomarkers, and ultimately how to prevent quality defects.

1. Application of proteomics to meat quality

Proteomics is the study of the proteome (i.e. a set of proteins) which contains information on gene expression and protein translation (Petracci & Cavani, 2012). Two-dimensional electrophoresis (2-DE) in combination with mass spectrometry (MS) has been applied in meat quality research to understand growth and development, postmortem metabolism, calpain's role in tenderness, protein biomarkers related to tenderness, and water holding capacity (WHC) (Bendixen, 2005; Bouley, Chambon, & Picard, 2004; Carvalho et al., 2014; Desai et al., 2016; Gorg, Weiss, & Dunn, 2004; Phongpa-Ngan, Grider, Mulligan, Aggrey, & Wicker, 2011; Picard et al., 2015). In addition, proteomics is applied for elucidating protein modifications such as reversible phosphorylation, oxidation, degradation, and denaturation in postmortem meat (Huang & Lametsch, 2013). Several researchers have also focused on understanding early postmortem protein changes using proteomics (D'Alessandro, Marrocco, Zolla, D'Andrea, & Zolla, 2011; Jia et al., 2007; Lametsch et al., 2003; Promeyrat et al., 2011; Wu, Fu, Therkildsen, Li, & Dai, 2015). Proteomics is an important tool in determining quality biomarkers that are indicators of meat quality defects (Laville et al., 2009; Marcos & Mullen, 2014; Rodrigues et al., 2017; van de Wiel & Zhang, 2007).

Application of proteomics for investigating meat quality is a relatively new approach (< 20 years old), but has been used to elucidate the relationship between the muscle proteome and the conversion of muscle to meat (Jia, Hildrum, et al., 2006; Jia et al., 2007; Lametsch et al., 2011; Morzel et al., 2004; Sayd et al., 2006), tenderness (Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006a; Lametsch et al., 2003; Laville et al., 2009), color (Canto et al., 2015; Joseph, Suman, Rentfrow, Li, & Beach, 2012; Nair et al., 2016; Yu et al., 2017), and water holding capacity (van de Wiel & Zhang, 2007).

2. Application of proteomics to beef quality

2.1. Proteome analysis of bovine muscle in postmortem period

Bouley et al. (2004) studied the mapping of bovine skeletal muscle using two-dimensional gel electrophoresis and mass spectrometry. They identified 129 protein spots corresponding to metabolism, cell structure, cell defense, and contractile apparatus. Jia, Hildrum, et al. (2006) analyzed the changes in enzymes associated with energy metabolism during the early postmortem period in *longissimus thoracis* bovine muscle. Twenty four metabolic and heat shock proteins changed in samples collected at different postmortem times. Jia, Hollung, et al.

* Corresponding author.
E-mail address: schilling@foodscience.msstate.edu (M.W. Schilling).

http://dx.doi.org/10.1016/j.meatsci.2017.04.018
Received 16 February 2017; Received in revised form 19 April 2017; Accepted 19 April 2017

(2006) conducted proteome analysis of two bovine muscle types: *M. longissimus dorsi* and *M. semitendinosus*, and reported that five proteins (cofilin, lactoylglutathione lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP 27, and HSP20) were affected during early post-mortem storage in both muscles. Similarly, Jia et al. (2007) examined the proteome changes up to 24 h postmortem in bovine *longissimus thoracis* muscle. Results demonstrated that 47 protein spots changed during the first 24 h postmortem. Application of 2DE in the study identified approximately 1000 individual protein spots.

2.2. Tenderness

Picard et al. (2015) reported 21 potential biomarkers for tenderness in beef, which included heat shock, metabolic, structural, oxidative resistant, and proteolytic proteins. In addition, Picard et al. (2015) discussed underlying mechanisms, biomarker discovery, biomarker evaluation, and validation. Gagaoua, Terlouw, Boudjellal, and Picard (2015) determined correlation networks among these protein biomarkers and reported that PRdx6 were correlated with Hsp20 ($r = 0.53$, $P < 0.01$) and μ-Calpain ($r = 0.49$, $P < 0.01$). These authors went on to explain that antioxidant proteins and heat shock proteins appear to play key roles in the development of tenderness. Franco et al. (2015) evaluated the effects of pre-slaughter stress on proteome changes in bovine *longissimus thoracis*. Ten proteins were differentially expressed in dark firm and dry (DFD) beef in comparison to normal beef. Seven of these proteins were structural-contractile and 3 proteins were metabolic in nature. These authors reported that highly phosphorylated fast skeletal myosin light chain 2 isoforms were more abundant in control beef in comparison to DFD meat and these were the most sensitive biomarkers that were detected for the incidence of DFD meat.

2.3. Color

Muscle-specificity in beef color stability was investigated by Joseph et al. (2012) using proteomic tools. These authors compared the sarcoplasmic protein profile of color-stable *longissimus lumborum* (LL) and color-labile *psoas major* (PM), and correlated the differentially abundant proteins with color traits. Metabolic enzymes (β-enolase and triose phosphate isomerase), antioxidant proteins (thioredoxin, peroxiredoxin-2, dihydropteridine reductase, aldose reductase, and peptide methionine sulfoxide reductase) and chaperones (heat shock protein-27 kDa, heat shock protein-1 B-70 kDa, and stress-induced phosphoprotein-1) were more abundant in color-stable LL compared with color-labile PM. Moreover, proteins having a positive correlation with redness (aldose reductase, creatine kinase, and β-enolase; $r = 0.64–0.72$) and color stability (peroxiredoxin-2, peptide methionine sulfoxide reductase, and heat shock protein-27 kDa; $r = 0.87–0.92$) were overabundant in LL, whereas the protein having negative correlation with redness was overabundant in PM (mitochondrial aconitase 2, $r = -0.59$). These findings indicated that the greater abundance of antioxidant and chaperone proteins in LL compared to PM was responsible for the greater color stability of LL. Previous research has indicated that antioxidants can improve meat color stability by limiting lipid oxidation and myoglobin oxidation (Renerre, Dumont, & Gatellier, 1996). Further, Wu et al. (2016) examined the sarcoplasmic proteome profile of LL and PM in Chinese Luxi yellow cattle, and reported differential abundance of several glycolytic and antioxidant proteins between the muscles.

Proteomic approaches have been utilized to examine the molecular basis of intramuscular variation in beef color stability (Nair et al., 2016). For example, beef semimembranosus is a large muscle in beef hindquarters exhibiting intramuscular differences in color stability. Steaks from the outside region (OSM) of the *semimembranosus* is color-stable during retail display, whereas steaks from the inside region (ISM) of the *semimembranosus* is color-labile. Due to the location in the carcass, OSM and ISM demonstrate variation in postmortem temperature decline and pH drop during carcass chilling which in turn could be contributing to the color stability variations (Sammel, Hunt, Kropf, Hachmeister, & Johnson, 2002). Proteome analysis indicated that the ISM steaks had greater abundance of glycolytic enzymes (fructose-bisphosphate aldolase A, phosphoglycerate mutase 2 and β-enolase) than their OSM counterparts. The increased levels of glycolytic enzymes indicated the possibility of a rapid pH decline in postmortem ISM, which in combination with its high temperature during carcass chilling could have an adverse effect on myoglobin redox stability (Faustman, Sun, Mancini, & Suman, 2010; Mancini & Hunt, 2005; Suman & Joseph, 2013; Suman, Nair, Joseph, & Hunt, 2016), thereby compromising the meat color stability.

Another factor that can cause variation in beef color stability of beef is the animal effect (King, Shackelford, & Wheeler, 2011). The biochemical basis of this animal-to-animal variation in color stability was examined using proteomic tools (Canto et al., 2015). These authors utilized beef LL from ten color-stable and ten color-labile carcasses for sarcoplasmic proteome analysis. Glycolytic enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase M2) were more abundant in color-stable steaks and had a positive correlation with redness and color stability. These glycolytic enzymes could have a positive impact on NADH regeneration, which in turn helps to stabilize meat color.

The effect of breed (Romagnola × Podolian, Podolian, and Friesian) and aging time (1, 7, 14, and 21 days) on color and sarcoplasmic proteome profile of beef *longissimus* muscles was also examined using proteomic tools (Marino et al., 2014). Aging increased lightness (L^* values), whereas redness (a^* values) varied with breed. The response to aging was also breed-specific, with steaks from the Podolian and Romagnola × Podolian bulls having a greater redness with aging, whereas redness of from Friesian young bulls were not affected by aging. Mass spectrometric analysis indicated that the abundance of several proteins (β-enolase, creatine kinase M-type, fructose-bisphosphate aldolase B, glyceraldehyde 3-phosphate dehydrogenase, triose-phosphate isomerase, glutathione S-transferase P and protein DJ-1) decreased during aging, whereas those of others (phosphoglycerate kinase 1, β-enolase, glyceraldehyde-3-phosphate dehydrogenase, fructose-bisphosphate aldolase B, creatine kinase M-type, adenylate kinase isoenzyme 1, peroxiredoxin-6, peroxiredoxin-2, superoxide dismutase, histidine triad nucleotide-binding protein) was influenced by breed type. The authors suggested that the proteome analysis could provide the basis for the development of protein markers for meat quality. A recent proteomic study (Clerens et al., 2016) of four muscles (LL, PM, *semitendinosus*, and *infraspinatus*) from New Zealand-raised Angus steers identified twenty-four protein spots with intensity differences, whereas peptidomic analysis identified forty-four peptides, indicating clear distinction between the proteome profile of muscles.

3. Application of proteomics to pork quality

In pigs, proteomics has been applied to evaluate postmortem protein degradation/modification, meat quality (PSE, WHC, pH), and meat color. Lametsch, Roepstorff, and Bendixen (2002) used proteomics (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) to identify protein degradation in *Longissimus dorsi* pig muscle. Nine different proteins including three structural proteins (actin, myosin heavy chain, and troponin T) and six metabolic proteins (glycogen phosphorylase, creatine kinase, phosphopyruvate hydratase, myokinase, pyruvate kinase, and dihydrolipoamide succinyltransferase) increased in concentration during postmortem storage with the exception of 43 kDa fragment of troponin T, which decreased in concentration over storage time. Lametsch et al. (2003) used proteomics to explain the postmortem changes in porcine muscle and its relationship to meat tenderness by measuring Warner-Bratzler shear force. Results from the study indicated that actin fragments (2, 6, 7), myosin heavy chain, myosin light chain II (MLC II), and triose phosphate isomerase I