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Application of proteomics to characterize and improve color and oxidative stability of muscle foods

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ABSTRACT

Meat and muscle foods are an integral part of human diet, and are becoming more relevant in resonance with the global population rise. Animal agriculture, in association with other related disciplines, is gearing up to meet this challenge utilizing rapidly evolving technologies. Consumer acceptability is a critical factor for muscle foods, and therefore quality, as well as quantity, of the animal-derived proteins is highly relevant. Proteomics, a relatively novel tool in animal science, could be utilized to comprehend the molecular basis of quality aspects in muscle foods including tenderness/texture, color, and functionality. Current review addresses the recent developments on the application of proteomics in meat and seafood quality with emphasis on color/appearance. Various proteomic tools employed are discussed as well as the applications are outlined including investigations on myoglobin structure and redox chemistry, and fresh meat color/color stability in beef, pork, chicken, and fish.

1. Introduction

Animal derived proteins play a significant role in human diet since ancient times and will continue that influence in future. The nutritional relevance of animal- or muscle food-derived proteins has been well recognized and documented (McAfee et al., 2010; McNeill, 2014). Therefore, as human population increases towards the estimated number of 9 billion by 2050 (Population Reference Bureau, 2015), efforts are underway to evaluate and implement novel technologies for meeting the increased global demand for high quality animal proteins. Accordingly, considerations should be given for technical advancements in animal agriculture addressing both pre-harvest and post-harvest phases, related to muscle food production and distribution. Proteomics is one example for application of cutting edge technology to explore the basic mechanisms related to quality aspects of meat/muscle-food production.

Proteome could be defined as "the protein complement of the genome which is comprised of the total amount of proteins expressed at a certain time point" (Wilkins et al., 1996). As a result, proteome is dynamic in nature compared to static genome, and influenced by various factors related to protein synthesis or degradation. Therefore, in perspective of meat science, proteome can be approached as a molecular linkage between the genetic composition and meat quality traits that are phenotypically expressed from the genome under different conditions (Hollung, Veiseth, Jia, Faergestad, & Hildrum, 2007); ante-mortem, peri-mortem, and post-mortem. Widely utilized in medical/pharmaceutical research, proteomic approach is relatively new to muscle food production and quality, with the past few years documenting a rapid growth in research publications related to proteomics of animal derived proteins. Early reviews by Bendixen (2005) and Mullen, Stapleton, Corcoran, Hamill, and White (2006) outlined the possibilities and utilization of proteomics tools in meat science, while the recent reviews by Paredi et al. (2013), D'Alessandro and Zolla (2013), and Almeida et al. (2015) summarized the research updates on proteomics of muscle food applications with reference to various meat species, including beef, pig, chicken, and fish. Furthermore, Ouali et al. (2013) focused on tenderness as a major meat quality attribute and published a comprehensive review on proteomic approach for exploring the mechanisms of beef tenderness. Along with tenderness, other quality traits such as color, water holding capacity, flavor, as well as proximate composition (lean/fat contents) are relevant to muscle foods.

As mentioned earlier, the global animal protein demand necessitates consideration and evaluation of all animal derived proteins/muscle foods. Among the several quality attributes of fresh meat, color/appearance is the most important one influencing consumer purchase decisions (Mancini & Hunt, 2005). Therefore, the objective of the

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present review is to summarize the advances in proteomics of muscle food quality traits with major focus on meat color and oxidative stability. Since meat/muscle food color is influenced by ante-, peri-, and post-mortem events, relevant recent research on post-mortem biochemistry in meat animal species utilizing proteomic tools is discussed.

2. Meat color

Meat color and appearance of muscle foods are critical to consumers with respect to acceptability at point of sale. For example, concerning red meats, consumers associate the bright cherry red color with freshness of meat and consider any change in color of meat from red to brown as the initiation of spoilage, although microbial safety may not have been compromised (Mancini & Hunt, 2005). The significance of meat color is underscored by the fact that annually there is a loss of $ 1 billion for the discounts of retail price for discolored meat in the US (Smith, Belk, Sofos, Tatum, & Williams, 2000). Myoglobin (Mb), the sarcoplasmic heme protein, is the major color pigment responsible for meat color, especially in red-meat species. The equilibrium between the redox forms of Mb determines the color of meat (AMSA, 2012; Mancini & Hunt, 2005). Deoxymyoglobin (DeoxyMb) is purple while oxymyoglobin (OxyMb) is cherry red in color and both these forms have iron in the heme group in reduced state. Metmyoglobin (MetMb) appears brown and has an oxidized iron (ferric) atom in heme. The color of freshly cut beef initially will be purple (DeoxyMb), which later oxygenates and form the bloomed color (cherry red) of OxyMb. Both DeoxyMb and OxyMb are oxidized into MetMb (Livingston & Brown, 1981) under the storage conditions resulting in the loss of red color. Other pigments such as hemoglobin and cytochrome C might also be relevant as pigment sources in aquatic species and avian meat species (AMSA, 2012; Mancini & Hunt, 2005).

3. Factors influencing meat color

The extrinsic and intrinsic factors which influence meat color were extensively reviewed by Mancini and Hunt (2005), and recently by Suman, Hunt, Nair, and Rentfrow (2014). The species of animal, breed (Faustman & Cassens, 1990), genetics (Lindahl et al., 2004), feeding systems and feed (Bruce, Stark, & Beilken, 2004), and husbandry practices (Lynch et al., 2002) affect meat color. In addition, animal handling practices (Channon, Payne, & Warner, 2000) and environment prior to slaughter have significant effect on meat color and can lead to conditions like PSE (pale, soft, exudative) and DFD (dark, firm, dry). The intrinsic factors including type of muscle fiber, location and function of muscle, concentration of Mb, and the metabolic process within the muscle (Hunt & Hedrick, 1977) also influence meat color. On the other hand, there are various post-harvest factors, such as packaging (Jakobsen & Bertelsen, 2000; Jayasingh, Cornforth, Carpenter, & Whittier, 2002; Seyfert, Mancini, Hunt, Tang, & Faustman, 2007), aging (Madhavi & Carpenter, 1993), antioxidants (Yin, Faustman, Riesen, & Williams, 1993; Hoving-Bolink, Eikelenboom, Van Diepen, Jongbloed, & Houben, 1998) and additives (Pohlman, Stivarius, McElyea, & Waldroup, 2002; Stivarius, Pohlman, McElyea, & Waldroup, 2002) that play a key role in determining meat color/color stability. Biochemical aspects of meat color and meat color stability have been well-documented by various researchers, with respect to meat species. The current review will focus on the utilization of proteomic tools to investigate color related quality attributes in muscle food.

4. Proteomic approaches in meat quality research

Proteomics encompasses separation, identification, and characterization of protein (Peng & Gygi, 2001) and utilizes either top–down or bottom–up approach. In general, the concept involves isolation of proteins from biological tissues/system (meat or muscle food in this case), and separation of proteins in electrophoretic gel-based methods, where the proteins get separated based on pH (first dimension), and molecular weight (second dimension), together represented as 2-DE (2-Dimensional Gel Electrophoresis, Fig. 1). The differently expressed proteins determined based on gel-image analysis (e.g. PDQEST), with potential correlation to a quality attribute, are then subjected to tryptic digestion, followed by mass-spectrometric analysis for identification. Proteins are characterized utilizing bioinformatics tools (e.g. Mascot, Protein Pilot) in conjunction with protein databases (e.g. Swiss-Prot, NCBI data base). This generally represents the work flow in top down approach and is followed in most of the proteomic research investigations related to meat or muscle food quality. On the other hand, bottom up is a gel-free approach, where the proteins, after tryptic digestion, are separated based on chromatographic approach and subjected to mass spectrometry (LC–MS/MS). The identification/characterization of proteins in such methods could be made through isotope (ITRAQ; isobaric tags for relative and absolute quantitation, Aggarwal, Choe, & Lee, 2006) or chemical labeling, label free approach such as protein arrays (Lee & Nagamune, 2004), or SELDI-TOF (Surface-enhanced laser desorption/ionization; Marcos et al., 2013).

Regarding analytical methods, 2-DE is the classical method and has been the work horse in proteomics studies related to animal science and meat science. Another variant of 2-DE is DIGE (Difference Gel Electrophoresis) where the proteins could be labeled with fluorescent dyes and the differential expression could be analyzed in a single gel itself, compared to comparison of separate gel images in 2-DE. Although more sensitive, DIGE has not been employed very often in meat quality-related proteomic research publications, mainly due to cost limitations. However, various staining approaches (Coomassie Blue, Silver staining, etc.) gave 2-DE a relatively universal acceptance. The limitations and advantages of various protein separation and mass spectrometric approaches in biological systems have been documented by Aebersold and Mann (2003), Westermeier and Naven (2002), and Yates (1998).

Advanced technique such as metalloproteomics (Baldassini et al., 2015) has been utilized to examine meat quality in Zebu cattle (*Bos indicus*, Nellore breed). Interestingly, this approach utilized traditional 2-DE followed by the characterization of calcium ions in protein spot utilizing X-ray fluorescence as well mass spectrometric (ESI–MS) determination of candidate proteins. Another concept utilized in tenderness related proteomics research was 'phosphoproteomics', where Anderson, Lonergan, and Huff-Lonergan (2014) investigated the relevance of phosphorylation, a major post-translational protein modification, in beef

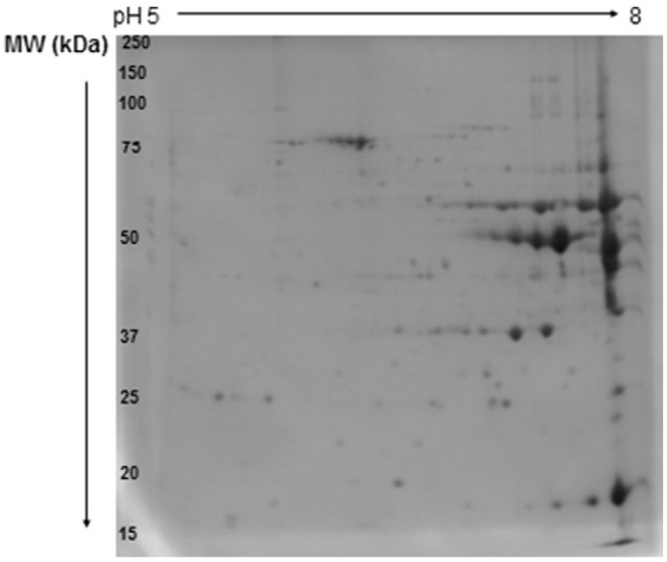

Fig. 1. 2-DE gel image of sarcoplasmic proteome from beef Longissimus lumborum (LL) muscle with pH range between 5 and 8 and molecular weight about 250 to 10 kDa. Adapted from Joseph et al., 2012, Journal of Agriculture and Food Chemistry, 60, 3196–3203.