

Differential aging-related changes in protein profiles of dark-cutting and normal-pH beef *longissimus lumborum* muscle

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Introduction: Dark-cutting beef is a meat quality defect associated with dark-meat surface color when the cut surface is exposed to oxygen. As a result, dark-cutting beef is discounted during grading due to its appearance. Previous studies have shown that dark-cutting beef has differential protein profiles than normal-pH beef. However, the effects of aging on dark-cutting muscle protein profile are not clear.

Purpose: The objective of the current research was to determine the extent to which protein expression profiles are altered with aging in dark-cutting *longissimus* muscles compared with normal-pH beef, and the associated impacts on meat surface color.

Methods: *Longissimus lumborum* (LL) muscles from 12 different animals (6 dark-cutters and 6 normal-pH beef) were collected within 48 h post-mortem, and sectioned into 2 equal sections randomly assigned to 7 and 14 days of aging, respectively. Following aging, muscle samples were subjected to quantitative proteomics analysis using LC-MS/MS-based proteomics. Collected data were analyzed using bioinformatics approaches, and the aging-related changes in protein expression profiles were considered significant at a false discovery rate (FDR) < 0.05.

Results: Mass spectrometry analysis identified 1000 proteins, of which 283 showed significant differential aging-related changes in protein expression profiles (FDR < 0.05) between dark-cutting and normal-pH beef. Of the 282, 95 were up-regulated, and 25 were down-regulated on aging day 7 (fold change >1.4, FDR < 0.05) in dark-cutting beef. In contrast, 25 proteins were up-regulated and 49 were down-regulated on day 14 (fold change >1.4, FDR < 0.05) in dark-cutting beef. Of the 25 up-regulated proteins in dark-cutting beef on day 14, 30% were involved in mitochondrial complex I activity. Conversely, proteins involved with energy metabolism such as glycolysis, glycogen degradation, glycerol-phosphate shuttle, stress, and heat shock proteins were down-regulated in dark-cutting beef on day 14.

Significance: Our results suggest that aging has muscle-specific effects on protein profiles. This study enables elucidation of the biochemical and molecular basis of dark-cutting induced color deviations.

Keywords: Aging, Meat color, Dark-cutting beef, Proteomics, Mass spectrometry