# IDENTIFICATION OF DIFFERENTIAL S-NITROSYLATED PROTEINS AND SITES IN DARK-CUTTING BEEF AND NORMAL BEEF USING ISOBARIC IODOTMT SWITCH ASSAY

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## I. OBJECTIVES

Dark-cutting beef is a typical representative of abnormal meat that has dark, firm, and dry characteristics. This study aimed to compare the difference of S-nitrosylated proteins between dark-cutting beef and normal beef in the early postmortem stage.

## II. MATERIALS AND METHODS

Animal and meat samples Simmental-cross cattle with average carcass weight of 341 ± 20 kg and age of 18 mo under the same raising and slaughter conditions were selected from a commercial abattoir (Hengdu Food Co., Ltd., Bi'yang, China). *Longissimus thoracis* (LT, 12th to 13th rib) were taken from the left side of the carcasses at 24 h postmortem. Based on the ultimate pH, samples were divided into normal beef group ($5.40 < pH_{24} \leq 5.80$, $n = 6$) and dark-cutting beef group ($pH_{24} \geq 6.09$, $n = 6$). Each group was added with 4 vol of lysis buffer (1% sodium dodecyl sulfate, 1% protease inhibitor cocktail, 50 mM iodoacetamide). The protein solution mixed with 6 time volume of acetone at −-20°C. The acetone-precipitated protein was redissolved in a solution (50 mM N-2-hydroxyethylpiperazine-N-ethane- sulphonicacid, 1 mM EDTA, 0.1% sodium dodecyl sulfate). The samples were labeled with iodo-tandem mass tags (iodoTMT126–-129). An immobilized anti-TMT resin was used to enrich S-nitrosylated proteins.
Statistical analysis: Relative quantification was performed based on the abundance of the reported ion of the tag, and it determined the proteins that were specific through UniProt Bovine (http://www.uniprot.org/). The different sites were screened with 1.2 times as the fold change, and the coefficient of variation was less than 0.1.

## III. RESULTS

A total of 856 S-nitrosylated sites from 257 proteins were identified in the current study. Among them, 803 sites on 240 proteins contained quantitative information as shown in Table 1. The number of available effective spectrograms was 4,509, and the utilization rate was 20.5% after the protein theory data database was searched. A total of 2,118 peptides and 670 S-nitrosylated peptides were identified by spectrogram analysis (Table 1). These results imply that a large number of proteins were endogenously S-nitrosylated in beef muscle. In dark-cutting beef samples, there were 131 modification sites upregulated, whereas 31 modification sites were downregulated based on the standard of fold change > 1.2 and coefficient of variation < 0.1 (Figure 1). Dark-cutting beef samples showed a higher number of S-nitrosylated proteins compared to normal beef samples, indicating that more cysteines and proteins were available for S-nitrosylation.

Table 1.
Basic statistical values of S-nitrosylated proteins from LC-MS/MS

| Total spectrogram number | Matching spectrogram number | Peptides | Modified peptides | Identification proteins | Quantification proteins | Identification sites | Quantitative sites |
|---|---|---|---|---|---|---|---|
| 21991 | 4509(20.5%) | 2118 | 670 | 257 | 240 | 856 | 803 |

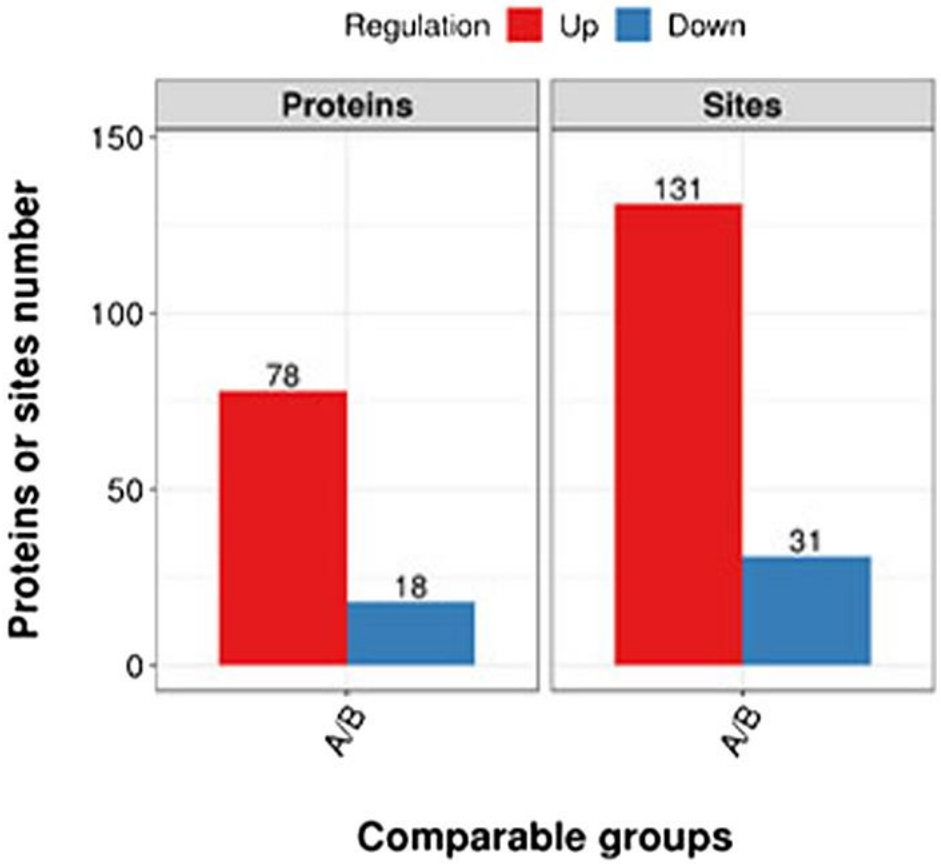


Figure 1.
Histogram of the number distribution of differential S-nitrosylated expressed proteins and modification sites in different comparison groups. (A represented dark cutting beef samples. B represented normal beef samples.)

## IV. CONCLUSION

This study identified and compared the differential S-nitrosylated proteins between dark-cutting beef and normal beef at 24 h postmortem. The *Longissimus thoracis* muscles were labeled with iodoTMT126–129 for the liquid chromatography-tandem mass spectrometry analysis. A total of 856 S-nitrosylated sites from 257 proteins were identified. The S-nitrosylated protein intensity in dark-cutting beef samples was higher compared to that of normal beef samples along with a large number of cysteine sites. Further studies should focus on the effects of protein S-nitrosylation on the variation of beef quality during postmortem aging.

Keywords: dark-cutting beef, iodo-tandem mass tags switch assay, S-nitrosylation