MITOCHONDRIAL PROTEOME CHANGES OF BEEF *LONGISSIMUSS LUMBORUM* MUSCLE DURING POST-MORTEM STORAGE

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I. INTRODUCTION

Color discoloration during meat storage is mainly caused by a reduction in metmyoglobin (MetMb) reducing activity, decreased NADH content and oxygen consumption rate. Mitochondria were reported to be involved in all of these pathways, based on an ability to provide the sties for electron transport chain [1] as well as being permeable to cytochrome c, and the capacity to yield NADH and to competitively consume oxygen against myoglobin. However, few studies have been conducted focusing on the roles that mitochondrial enzymes play in meat color development during post-mortem storage. The objective of this study was to explore the changes in key mitochondrial proteins of beef *longissimuss lumborum* muscle during postmortem storage via differential mitochondrial proteomics analysis.

II. MATERIALS AND METHODS

Chinese crossbred yellow cattle (24 months old, n=3) carcasses were randomly selected on the slaughter line at grading in a local abattoir. The M*. longissimus lumborum* (LL) were removed from the left side, packaged and transferred to the lab (0-4°C). The LL were portioned into 2.54 cm thick steaks, vacuum packaged and stored at 2 ± 2 °C. Samples for mitochondria proteomics analysis were taken from 3 steaks at day 0, 4, 7, 14 and 21 of storage, and kept at -80 °C. Then the mitochondria were isolated and proteins were extracted. The mitochondrial proteome was analyzed employing two-dimensional electrophoresis. The gels were stained, and the images were analyzed to determine differences in the abundance of protein spots. The proteins spots exhibiting 1.5-fold or more intensity differences ($P < 0.05$) between the storage points were identified by tandem mass spectrometry.

III. RESULTS AND DISCUSSION

Eleven spots were successfully identified as 9 mitochondrial proteins, of which 6 were over abundant as storage time extended (Table 1). DJ-1 protein (PARK7) and Gluthathione S-transferase class pi (GSTP1) both play important roles in cell protection against oxidization. ATP-specific succinyl-CoA synthetase represents the only step of substrate-level phosphorylation in the TCA and succinyl-CoA ligase [ADP-forming] subunit beta (SCCLA2) provides nucleotide specificity of the enzyme and binds the substrate succinate. At 21d of storage, PARK7, GSTP 1 and SUCLA2 were over expressed a response to oxidative stress; those proteins have been reported to be negatively related with beef *a** value [2,3,4]. While voltage-dependent anion channel 1 (VDAC1) acts as a scaffold for many proteins and allows for the flux of ions and metabolites (i.e. ATP) through interactions within the pore; it`s expression increased 6 fold during early storage, but the fold changes were kept at the same level through out aging. VDAC1 was seldom observed in previous studies, and its relationship with meat color requires further verification, as well as Tu translation elongation factor (TUFM). Isocitrate dehydrogenase subunit alpha (IDH3A), is the catalytic subunit of the enzyme which catalyzes the decarboxylation of isocitrate into alpha-ketoglutarate, which was found to be over abundant in color labile beef muscles [5]. Three proteins exhibited lower expression relative to day 0 samples. They were Chain A, Subcomplex of the stator of bovine mitochondrial ATP synthase (ATP5F1), Acyl-CoA synthetase long-chain family member 1(ACSL1), Chain A, Native bovine skeletal calsequestrin, low-Ca^{2+} form (CASQ1) (Table 1). Similar with previous findings, the ATP5F1 was down regulated with extended storage, and is also reported to be correlated positively with *a** value [4]. The Acyl-CoA synthetase

is involved in the TCA and related to the production of ATP. The calcium-binding protein (CASQ1), acts as an internal calcium store in muscle; it exhibited a large decrease during storage (Table 1).

Table 1 Differentially abundant mitochondrial proteins in beef LL steaks during 21 days chilled storage

Identified proteins	Gene name	Theoretical pI/MW(kDa)	SC[a]	MS[b]	Fold changes				Molecule Function
					4d	7d	14d	21d	
voltage-dependent anion channel 1	VDAC1	8.62/30.836	71%	883	↑ 6.0	↑ 5.3	↑ 6.6	↑ 7.7	Porin
Tu translation elongation factor	TUFM	6.72/49.709	23%	330	↑ 4.1	↑ 5.9	↑ 9.1	↑ 7.3	GTP- binding
DJ-1 protein	PARK7	6.84/20.194	24%	113	↑ 6.2	↑ 2.7	-	↑ 17.8	antioxidant
gluthathione S-transferase, class pi	GSTP1	6.89/23.826	69%	749	-	-	-	↑ 4.1	antioxidant
succinyl-CoA ligase [ADP-forming] subunit beta,	SUCLA2	6.73/50.456	14%	261	-	-	-	↑ 3.4	Ligase
succinyl-CoA ligase [ADP-forming] subunit beta	SUCLA2	6.73/50.456	10%	238	-	-	-	↑ 3.9	Ligase
isocitrate dehydrogenase [NAD] subunit alpha	IDH3A	6.51/40.100	28%	415	-	-	-	↑ 2.9	NAD binding
Chain A, subcomplex of the stator of bovine mitochondrial ATP synthase	ATP5F1	9.14/24.710	39%	355	↓ 2.3	↓ 7.4	↓ 4.03	-	Metabolic
Chain A, subcomplex of the stator of bovine mitochondrial ATP synthase	ATP5F1	9.14/24.710	39%	238	↓ 3.2	↓ 14.1	↓ 12.4	↓ 3.5	Metabolic
Acyl-CoA synthetase long-chain family member 1	ACSL1	6.87/79.200	12%	150	↓ 3.0	↓ 4.8	↓ 5.34	↓ 10.0	Metabolic
Chain A, native bovine skeletal calsequestrin, low-Ca^{2+} form	CASQ1	3.97/41.495	32%	398	↓ 5.0	↓ 84.1	↓ 12.4	↓ 839.3	calcium ion binding

[a]Sequence coverage. [b]Mascot score. ↓, ↑ lower or higher expression ($P < 0.05$) relative to day 0 samples. "-" the fold changes were not significant ($P > 0.05$)

IV. CONCLUSION

It is well known that beef color and color stability decreases as storage time is prolonged. From this study, it indicates that meat discoloration maybe due to the increased oxidation process of the beef muscle during storage, which caused the up-regulation of antioxidant mitochondrial proteins, including PARK7 and GSTP1, and the oxidation process may indirectly affect the MetMb reducing ability. While the down-regulated metabolic proteins, such as ATP5F1 and ACSL1, indicate a decreased metabolic ability and possible reduced oxygen consumption of beef during storage. The analysis of mitochondrial proteins of this study narrowed the scope for screening potential color biomarkers for the antioxidant and ATP metabolic mitochondrial proteins. However, further exploration is required to verify the direct relationship between those proteins and beef color traits.

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REFERENCES

1. Tang, J., Faustman, C., Mancini, R. A., Seyfert, M., & Hunt, M. C. (2005). Mitochondrial Reduction of Metmyoglobin: Dependence on the Electron Transport Chain. Journal of Agricultural and Food Chemistry 53: 5449-5455.
2. Wu, W., Gao, X., Dai, Y., Fu, Y., Li, X., & Dai, R. (2015). Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in M. *semitendinosus* of Chinese Luxi yellow cattle. Food Research International 72: 98-105.
3. Wu, W., Yu, Q., Fu, Y., Tian, X., Jia, F., Li, X., & Dai, R.(2016). Towards muscle-specific meat color stability of Chinese Luxi yellow cattle: A proteomic insight into post-mortem storage. Journal of Proteomics 147:108-118.
4. Mancini, R. A., Suman, S. P., Nair, M. N., Belskie, K., Li, S., Beach, C. M., & Ramanathan, R. (2016). Differential abundance of mitochondrial proteome in beef muscles. Meat Science 112: 169.
5. Yu, Q., Wu, W., Tian, X., Jia, F., Xu, L., Dai, R., & Li, X. (2017). Comparative proteomics to reveal muscle-specific beef color stability of Holstein cattle during post-mortem storage. Food Chemistry 229: 769-778.