Proteomics of Meat Color

Surendranath P. Suman, Ph.D.
Assistant Professor
Animal and Food Sciences
Meat Color

• Influence consumers’ decisions
  – Sight sells!

• Meat discoloration = Sales loss
  – Annual sales loss $1 billion (Smith et al., 2000)

• Myoglobin chemistry
Interinfluential Interactions

- Antioxidants
- Lipid oxidation
- Ligands
- Sarcoplasmic proteome

Mancini & Hunt, 2005, Meat Science
Application of Proteomics

• “Why” and “How”
• Mb and biomolecules
  – Prooxidants
  – Antioxidants
• Muscle-specificity in beef color
  – Role of sarcoplasmic proteome
• Species differentiation
Lipid oxidation-induced meat discoloration

OxyMb  MetMb

O₂

Reactive Lipid Oxidation Products

Vitamin E

TQ  TQE₁

THQ  TQE₂
Vitamin E and Meat Color

Lipid Oxidation  ↓  Color Stability

• Species-specificity in meat color
• Myoglobin sequence affects color stability
4-Hydroxy-2-nonenal (HNE)

- α, β- Unsaturated Aldehyde
  - Oxidation of ω-6 PUFA
  - Alters protein functionality
  - Detected in fresh meats

- Adduction in Mb
  - Histidine (Alderton et al., 2003; Lee et al., 2003)
  - HIS 93 and 64
  - Heme stability

- Histidines in Mb
  - Beef = 13
  - Pork = 9
MetMb formation in beef and pork myoglobins incubated with HNE at pH 5.6, 4°C

- **Beef Mb**
- **Pork Mb**

The graph shows the percentage of MetMb formation over time for beef and pork myoglobins incubated with HNE at pH 5.6, 4°C. The data is presented at 0 h and 48 h, with error bars indicating variability.
Pork Mb + HNE at pH 5.6, 4°C, 72 h

Voyager Spec #1 => BC => NF0.7 => MC [BP = 16954.8, 20991]

16956.25 = Mb

17114.34 = Mb:1HNE (Mb+158)
Beef Mb + HNE at pH 5.6, 4°C, 72 h

Voyager Spec #1 => BC => NF0.7 => MC [BP = 16940.5, 36858]

- Mb:2HNE (Mb+314) at 17254.01
- Mb:1HNE (Mb+157) at 17097.45
- Mb at 16940.63

Mass (m/z) vs % Intensity graph.
HNE adduction sites in Pork Mb

Red: Adducted
Green: Unadducted
HNE adduction sites in Beef Mb

- HIS-152
- HIS-93
- HIS-88
- HIS-81
- HIS-36
- HIS-24
- HIS-119
PIC-labeling Quantitation

RT: 22.00 - 28.00

PIC-H*LAESHANK
Bovine Mb peptide 88-96

Base Peak
ms2 642.34

Base Peak
ms2 645.34

$^{13}$C$_6$PIC-H*LAESHANK
Bovine Mb peptide 88-96
Kinetics of HNE adduction in Pork Mb

Single Phase Exponential Association

\[ Y = Y_{\text{max}} \cdot (1 - \exp(-K \cdot X)) \]

Observed $K$ value (min\(^{-1}\))

HIS-36

3.6 $\times$ 10\(^{-4}\)
Kinetics of HNE adduction in Beef Mb

Single Phase Exponential Association

$$Y = Y_{\text{max}} \times (1-\exp(-K \times X))$$

Observed $K$ values (min$^{-1}$)

- HIS-81: $7.4 \times 10^{-3}$
- HIS-88: $14.5 \times 10^{-3}$
Species-specific meat discoloration

• Beef Mb is more susceptible than pork Mb
  – HIS 93 adduction
  – Adduction near heme pocket

• Lipid oxidation is critical to beef color

Suman et al., 2007, Proteomics
Lactate-Mb Interactions

• Color stabilizer

• How does lactate stabilize meat color?
  – Direct interactions with Mb
  – Indirect interactions with enzymes

• Direct interactions with Mb (Giardina et al., 1996)
  – Adduct formation?

• Species-specificity (Tamburrini et al., 1999)
  – Modulated in horse and sperm whale Mb
  – Not in Emperor penguin Mb

• MALDI-TOF MS
OxyMb incubated with lactate

OMb control
Incubation: 0 h

OMb + lactate
Incubation: 0 h

OMb control
Incubation: 192 h

OMb + lactate
Incubation: 192 h

Mancini et al., 2010, Meat Science
Static Genome Vs. Dynamic Proteome
Muscle-specificity in Beef Color

• **Filet Mignon**
  – Psoas major (PM)
  – Tender and expensive
  – Color-labile

• **NY Strip Steak**
  – Longissimus lumborum (LL)
  – Tougher and less expensive
  – Color-stable

• Response to MAP
• Cooked color
MAP and muscle source affect raw $a^*$ value

Packaging type  

Mancini et al., 2009, Meat Science
Muscle source influences internal cooked color

Suman et al., 2009, Meat Science
Beef Muscle Profiling

- **Muscle specificity in color**
  
  \[(McKenna \textit{et al.}, 2005)\]
  
  - Lipid oxidation, Oxygen consumption
  - OxyMb oxidation, MetMb reduction

- **Sarcoplasmic proteome**
  
  - 30% of muscle proteins
  - Proteins and enzymes interact with Mb
  - Differential abundance in LL vs. PM
DIGE - Sarcoplasmic Proteome

Green = LL
Red = PM
Yellow = STD
Differential Abundance of Proteome

• Identify and quantify proteome
  – 2-DE, MS-MS, peptide mass fingerprinting

• Beef muscles: LL and PM
  – Seven carcasses (n = 7)
  – 24 h post-mortem; 2.54 cm steaks
  – Retail display (0, 5, and 9 days)
  – Proteome samples at 0 day

• Meat color attributes
  – $L^*$ (darkness), $a^*$ (redness), $b^*$ (yellowness)
  – R630nm/580nm; Metmyoglobin Reducing Activity (MRA)

• Correlate with color attributes
  – Protein markers for meat quality

USDA Grant 2009-35503-05194
Redness ($a^*$ value) and muscle source

![Graph showing the relationship between redness ($a^*$ value) and muscle source over days. The graph compares two lines, one for LL and another for PM, showing a decrease in redness over time.](image-url)
R630/580 and muscle source

![Graph showing the change in R630/580 over days for LL and PM muscle sources.](Image)
MRA and muscle source
Proteome Analysis

- **2-Dimensional Electrophoresis**
  - pH 5-8, 17 cm IPG strips
  - Isoelectric focusing (First dimension)
  - 12% SDS-PAGE (Second dimension)
  - Staining and imaging

- **Gel analysis**
  - PDQUEST
  - Intensity difference = 1.5 fold or more

- **Tandem MS**

- **NCBI database**
pH 5-8; 17 cm; 12% gel
# Differentially Abundant Proteins

## Over-abundant in LL

<table>
<thead>
<tr>
<th>Spot #</th>
<th>Description</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2604</td>
<td>Pyruvate dehydrogenase (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>5403/6404</td>
<td>Creatine kinase (M chain) (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>0104</td>
<td>Peroxiredoxin-2 (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>4201</td>
<td>Triose phosphate isomerase (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>1001</td>
<td>Phophohistidine phosphatase (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>2702</td>
<td>Heat Shock Protein-70 KDa (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>2201</td>
<td>Heat Shock Protein-27 KDa (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>5204</td>
<td>Dihydropteridine reductase (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>4202</td>
<td>Peptide methionine sulfoxide reductase (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>3404</td>
<td>β-enolase (P &lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>0002</td>
<td>Thioredoxin (P &lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>3402</td>
<td>Aldose reductase (P &lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>3702</td>
<td>Stress-induced phosphoprotein-1 (P &lt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>

## Over-abundant in PM

<table>
<thead>
<tr>
<th>Spot #</th>
<th>Description</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3802</td>
<td>Serotransferin (P &lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>8803</td>
<td>Mitochondrial aconitase (P &lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>2103</td>
<td>Protein DJ (P &lt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>
## Correlation with Color Traits

<table>
<thead>
<tr>
<th>Protein</th>
<th>Muscle</th>
<th>Trait</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldose reductase</td>
<td>LL</td>
<td>$a^*$ value</td>
<td>+ 0.64</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>LL</td>
<td>$a^*$ value</td>
<td>+ 0.72</td>
</tr>
<tr>
<td>β-enolase</td>
<td>LL</td>
<td>$a^*$ value</td>
<td>+ 0.64</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase</td>
<td>LL</td>
<td>$a^*$ value</td>
<td>+ 0.65</td>
</tr>
<tr>
<td>Mitochondrial aconitase</td>
<td>PM</td>
<td>$a^*$ value</td>
<td>– 0.59</td>
</tr>
<tr>
<td>Peroxiredoxin-2</td>
<td>LL</td>
<td>R630/580</td>
<td>+ 0.92</td>
</tr>
<tr>
<td>Stress-induced phosphoprotein-1</td>
<td>LL</td>
<td>R630/580</td>
<td>+ 0.75</td>
</tr>
<tr>
<td>Heat shock protein-27kDa</td>
<td>LL</td>
<td>R630/580</td>
<td>+ 0.87</td>
</tr>
<tr>
<td>Peptide methionine sulfoxide reductase</td>
<td>LL</td>
<td>R630/580</td>
<td>+ 0.88</td>
</tr>
<tr>
<td>Peptide methionine sulfoxide reductase</td>
<td>LL</td>
<td>MRA</td>
<td>+ 0.63</td>
</tr>
</tbody>
</table>

*Poulson Joseph, 2011, PhD Dissertation, University of Kentucky*
Protein Categories

• Antioxidant proteins
  – Peroxiredoxin-2, Thioredoxin
  – Dihydropteridine reductase
  – Peptide methionine sulfoxide reductase

• Chaperone proteins
  – Heat Shock Protein-27 kDa
  – Heat Shock Protein-70 kDa
  – Stress-induced phosphoprotein-1

• Energy metabolism enzymes
  – Creatine kinase, β-enolase
  – Aldose reductase, Triose phosphate reductase

• Binding proteins
  – Serotransferin
Implications in Beef Industry

- Processing strategies
  - Aging
  - Packaging
  - Antioxidants
  - Enhancement

- Dietary manipulation
  - Antioxidant status

- Breed differences
  - Genetic selection
  - Brahman Vs. European cattle
Meat Species Differentiation

- Accurate mass detection
- MS coupled with
  - Collision-induced dissociation
    
  
    *(Ponce-Alquicira & Taylor, 2000)*
  - Edman degradation
- Mb species-specific sequence
- Emerging species’ Mb
  - Bison *(Joseph et al., 2009)*
  - Goat *(Suman et al., 2009)*
  - Emu *(Suman et al., 2010)*
  - Turkey *(Joseph et al., 2011)*
Where we go now?

- 1 protein
- 2 species
- 2 muscles
Prospective Applications

- Proteome profile of beef muscles
  - Proteome maps
- Effect of processing
  - Aging, packaging
- Color defects in fresh meats
  - DFD, PSE
  - Dark cutters
- Color defects in cooked meats
  - Persistent pinking in turkey
  - Premature browning in beef
Acknowledgments

University of Kentucky
• Poulson Joseph
• Shuting Li
• Mahesh Nair
• Dr. Youling Xiong
• Dr. Gregg Rentfrow
• Dr. Carol Beach

University of Connecticut
• Dr. Cameron Faustman
• Dr. Richard Mancini
• Ranjith Ramanathan
• Murali Konda

AMSA Family

Funding
• USDA AFRI
• University of Kentucky
• Kentucky Beef Council
Thank You

Questions?