Aging of different horse muscles: a proteomic approach

Pasquale De Palo, Rosaria Marino, Jose Manuel Lorenzo, Aristide Maggiolino
• Accepted by consumers as a natural and safe process

• Effective in increasing tenderness in meat from other species

• Easy to apply in groceries and butcheries

**AGING**

• Increases production costs and, so, final price

• Specific rules are lacking at European and national level


---

....taking the first steps...

- 4-5 days
- 30 hours
- 6-14 days
- 6-14 days
- 4 days
...a hot topic...

2006
- Seong, Lee, Park, Hah, & Ko. Meat quality and sensory characteristics in *longissimus muscle* of Jeju horse as influenced by ageing. *Journal of Animal Science and Technology.*

2014

2018
- Kaić, Žgur, Luštrek, & Potočnik. Physicochemical properties of horse meat as affected by breed, sex, age, muscle type and aging period. *Animal Production Science.*
- Ruiz, Beriain, Insausti, Lorenzo, Cantalejo, & Sarriés, Ageing effect on foal meat preservation. ITEA.

2019
- Maggiolino, Lorenzo, Marino, Della Malva, Centoducati & De Palo. Foal meat volatile compounds: effect of vacuum ageing on semimembranosus muscle. *Journal of the science of food and agriculture.*
How fibrillar proteolitic patterns change during aging in horse meat?

Is there any difference between muscle in proteolysis dynamics in horse carcass?

How protelysis affects meat tenderness in horse meat?
Materials and Method
Lab Analysis

- WBSF on grilled sample
- SDS – PAGE (Marino et al., 2013)
- Hydroxyproline and total collagen (Hutson et al., 2003)
- Tn-T Western blot (Marino et al., 2015)
- Myofibrillar fragmentation index (MFI) (Culler et al., 1978)
- 2 Dimensions Gel Electrophoresis (Marino et al., 2015)
Proc GLM SAS, 2013: Aging time, muscle, aging time x muscle and RRE as fixed effects

Fisher’s LSD test SAS, 2013: *post hoc* analysis
Results

**WBSF**

![WBSF graph](chart1.png)

**MFI**

![MFI graph](chart2.png)

**Total Collagen**

![Total Collagen graph](chart3.png)

- a, b, c, d = P < 0.05 in the row (aging effect)
- x, y, z = P < 0.05 in the column (muscle effect)
Results

- MIHC
- α-act
- Desmin
- Actin
- TnT
- TPM
- MLC1
- TnI
- TnC
- MLC2
a, b = P < 0.05 in the row (aging effect)
x, y, z = P < 0.05 in the column (muscle effect).
Results

**Desmin**

- a, x = P < 0.05 in the row (aging effect)
- y = P < 0.05 in the column (muscle effect)

**55 - 47 kDa**

- a, y = P < 0.05 in the row (aging effect)
- b, z = P < 0.05 in the column (muscle effect)

**Actin**

- a = P < 0.05 in the row (aging effect)
- b = P < 0.05 in the column (muscle effect)
Results

**TnT**

- **a, x**
- **a, y**
- **b**
- **b**
- **c**

**TPM**

- **x**
- **y**
- **z**

**30-28 kDa**

- **a**
- **b, x**
- **c, x**

\[ a, b, c = P < 0.05 \text{ in the row (aging effect)} \]
\[ x, y, z = P < 0.05 \text{ in the column (muscle effect)} \]
Results

MLC1

TnI

TnC

a, b = P < 0.05 in the row (aging effect)
x, y = P < 0.05 in the column (muscle effect).
Results

a, b = P < 0.05 in the row (aging effect)
x, y = P < 0.05 in the column (muscle effect).
<table>
<thead>
<tr>
<th></th>
<th>LLC</th>
<th>LLC</th>
<th>LLC</th>
<th>LLC</th>
<th>SM</th>
<th>SM</th>
<th>SM</th>
<th>SM</th>
<th>ST</th>
<th>ST</th>
<th>ST</th>
<th>SEM</th>
<th>Effects, P</th>
<th>Muscle</th>
<th>Aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact forms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38 kDa</td>
<td>21.4a</td>
<td>5.6b</td>
<td>2.4c</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>10.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.97</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>36 kDa</td>
<td>22.1</td>
<td>ND</td>
<td>ND</td>
<td>58.0a</td>
<td>11.0b</td>
<td>5.0c</td>
<td>38.7a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.61</td>
<td>***</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>34 kDa</td>
<td>25.8a</td>
<td>23.3b</td>
<td>21.2c</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.52</td>
<td>***</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Degraded forms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 kDa</td>
<td>22.7b</td>
<td>51.7a</td>
<td>46.7a</td>
<td>42.0b</td>
<td>89.0a</td>
<td>84.4a</td>
<td>25.2c</td>
<td>57.9b</td>
<td>100.0</td>
<td>a</td>
<td>1.83</td>
<td>**</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 kDa</td>
<td>8.0c</td>
<td>19.4b</td>
<td>29.6a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.72</td>
<td>***</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

ND= not detected; *= P<0.05; **=P<0.01; ***=P<0.001. a, b, c=P<0.05 in the row (aging effect).
Main outcomes....

Differences in WBSF between muscles and during aging time are not due to collagen content, but to myofibril degradation.

Between muscles there is variability in isoforms, concentration, degradation rate.

As in beef, interesting proteomic markers of tenderness could be Tn-T and MHC.

Muscles, independently from the collagen content, tend to increase tenderness during aging, in relation to proteolysis.
Thank you