Protein Carbonyls in Meat Systems: a Review

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1/ INTRODUCTION

2/ PROTEIN CARBONYLATION: MECHANISMS

3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

5/ FINAL REMARKS
1/ INTRODUCTION

PROTEIN OXIDATION

LACK OF KNOWLEDGE ON BASIC FOOD CHEMISTRY

LIPIDS
- LIPOLYSIS
  - FATTY ACIDS
  - OXIDATION
    - LIPID OXIDATION PRODUCTS

PROTEINS
- PROTEOLYSIS
  - PEPTIDES
    - AMINO ACIDS
  - OXIDATION
    - PROTEIN OXIDATION PRODUCTS

CARBOHYDRATES
- GLYCOLYSIS
  - SUGARS
  - MAILLARD REACTION
    - MAILLARD PRODUCTS

MEAT SCIENCE & TECHNOLOGY
LATE START (90s)
EMERGING TOPIC

PROTEIN OXIDATION
QUALITY

SCIENTIFIC AND TECHNOLOGICAL SIGNIFICANCE OF
PROTEIN OXIDATION

LIMITED REFERENCES TO PROTEIN OXIDATION
NO MENTION TO PROTEIN CARBONYLATION
WHY ARE MEAT PROTEINS OXIDIZED?

REACTIVE OXYGEN SPECIES

QUALITY TRAITS
- Eating quality traits
- Technological properties
- Nutritional value

P/C PROPERTIES
- Decreased solubility
- Increased hydrophobicity
- Increased aggregation
- Altered susceptibility to proteolysis
- Impaired functionality

CHEMICAL CHANGES
- Modification of amino acid side chains
- Cleavage of the hydrocarbon chain
- Formation of intra- & inter-molecular cross-links

Minireview
"Protein Oxidation in Aging, Disease, and Oxidative Stress"
Barbara S. Berlett and Karl R. Stadtmann
Protein carbonylation is the most remarkable change in oxidized proteins (Stadman & Levine, 2003).

Protein carbonyls are easily quantified in food samples.

- Total amount of carbonyls is employed as a general index of protein oxidation.

**DNPH method**

1. Assess P-OX during processing/storage

2. Effectiveness antioxidant treatments against P-OX

**INFORMATION OF LIMITED VALUE**

- No chemical structure
- No oxidation mechanism
- No info on source

**QUESTION**

- Which is the impact of carbonylation on meat products?
- Should we apply antioxidant strategies against P-OX?
**1/ INTRODUCTION**

- **Specific Protein Carbonyls**
  - **Daneshvar et al. 1997**
    - "First description of AAS/GGS as protein oxidation markers"
  - **Akagawa et al. 2006**
    - "Derivatization procedure with p-amino benzoic acid"

- **Fluorescent HPLC for the detection of specific protein oxidation carbonyls – α-aminoadipic and γ-glutamic semialdehydes – in meat systems**
  
  
  - **AAS**
    - alpha-aminoadipic semialdehyde
  - **GGS**
    - gamma-glutamic semialdehyde
  - **TOTAL CARBONYLS DNPH**
    - 70% GGS + AAS

- **Which is the impact of carbonylation on meat products?**
- **Should we apply antioxidant strategies against p-Ox?**
1/ INTRODUCTION

SPECIFIC PROTEIN CARBONYLS

CHEMISTRY BEHIND P-OX

DEFINED CHEMICAL STRUCTURES

PRECISE OXIDATION PATHWAYS

SPECIFIC OXIDATION MECHANISMS

IMPLICATION OF PROTEIN CARBONYLS
IN FURTHER REACTIONS AND CONNECTION
WITH OTHER BIOCHEMICAL REACTIONS

IDENTIFY POTENTIAL IMPACT OF
PROTEIN CARBONYLATION
ON PROTEIN FUNCTIONALITY
AND PARTICULAR MEAT QUALITY TRAITS

IDENTIFY NECESSITY OF
ANTIOXIDANT ACTIONS

DEVELOPMENT OF EFFECTIVE
ANTIOXIDANT STRATEGIES
1/ INTRODUCTION

2/ PROTEIN CARBONYLATION: MECHANISMS

3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

5/ FINAL REMARKS
2/ PROTEIN CARBONYLATION: MECHANISMS

FORMATION OF CARBONYLS IN PROTEINS

1. OXIDATION SIDE-CHAIN (Requena et al., 2001)
   - LYSINE
   - PROLINE
   - ARGinine

2. NON-ENZYMATIC GLYCATION (Akagawa et al., 2005)
   - GLUCOSE
   - LYSINE

3. CLEAVAGE OF PEPTIDE BACKBONE (Garrison, 1987)
   - α-AMIDATION PATHWAY
   - GLUTAMYL PEPTIDE
WORTH NOTING THAT...
Carbonylation involves the loss of protonable amino groups
Carbonylation occurs in protein-bound amino acids
Carbonylation requires the presence of transition metals and reactive water
Carbonylation occurs in the absence of oxidizing lipids
PROTEIN CARBONYLS (AAS/GGS) ARE NOT FINAL OXIDATION PRODUCTS
2/ PROTEIN CARBONYLATION: MECHANISMS

REACTIVITY OF PROTEIN CARBONYLS

A

AAS
O=NH

ADDITIONAL OXIDATIVE DEGRADATION

H₂O₂

H₂O

α-amino adipic acid

AAS
O=NH

B

CARBONYL MOIETY
STRECKER DEGRADATION

AAS
O=NH


C

AAS
O=NH₂

TRANSAMINATION

H₂O

CO₂

STRECKER ALDEHYDE

WORTH NOTING THAT...
Severe chemical modification
Impact on electric arrangement

WORTH NOTING THAT...
Intra-/inter-molecular CROSSLINK

WORTH NOTING THAT...
Strecker aldehydes are potent odorants
A previous proteolysis is required

RELIABLE INDICATOR OF P-OX (Sell et al., 2008)
2/ PROTEIN CARBONYLATION: MECHANISMS

CARBONYLATION OF MYOFIBRILLAR PROTEINS

BINDING TO PROTEINS
CAGED OXIDATIVE ACTION
(Hawkins & Davies, 1997)

MAIN PROMOTERS
OF CARBONYLATION
IN MEAT SYSTEMS

Cu⁺/Cu²⁺

Fe²⁺/Fe³⁺
Mb

SENSITIVITY OF PROTEINS
TO CARBONYLATION

AMINO ACID COMPOSITION

TERTIARY STRUCTURE

IN VITRO OXIDATION MYOFIBRILLAR PROTEINS (37º C)

IN VITRO OXIDATION PROTEINS (Fe/H₂O₂/37º C / 14 days)
CARBONYLATION IN MEAT AND MEAT PRODUCTS

IN VIVO \( \approx 0.7-1 \) nmol carbonyls/ mg proteins

AGING/STORAGE

PH DECLINE
- EXPOSURE TO AIR
- RELEASE OF PROOXIDANTS
- LIPID OXIDATION

PROCESSING
- COOKED PATTIES
- FRANKFURTERS
- DRY CURED PRODUCTS
- FERMENTED SAUSAGES
- FROZEN MEATS

MINCING
- HIGH TEMPERATURES
- SALTING
- HYDROSTATIC PRESSURE
- RADIATION

PROTEIN CARBONYLATION
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

PROTEIN FUNCTIONALITY

SENSORY PROPERTIES

NUTRITIONAL VALUE
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

PROTEIN FUNCTIONALITY

HYPOTHESIS

FORMATION OF PROTEIN CARBONYLS

LOSS OF PROTONABLE AMINO GROUPS

FORMATION OF CARBOXYLIC MOIETIES
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

**PROTEIN FUNCTIONALITY**

- Non-polar residues

**HYPOTHESIS**

**FORMATION OF PROTEIN CARBONYLS**
- Loss of protonable amino groups
  - Formulation of carboxylic moieties
  - Impaired electric arrangement
  - Altered water-protein interaction
    - Loss solubility
  - Altered intra-protein interactions
    - Unfolding
    - Denaturation
  - Increased aggregation

**IMPAIRED FUNCTIONALITY**
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

PROTEIN FUNCTIONALITY

- AMINO ACID COMPOSITION
- NATIVE STRUCTURE

HYPOTHESIS

FORMATION OF PROTEIN CARBONYLS

- LOSS OF PROTONABLE AMINO GROUPS
- FORMATION OF CARBOXYLIC MOIETIES
- IMPAIRED ELECTRIC ARRANGEMENT
- ALTERED WATER-PROTEIN INTERACTION
  - LOSS SOLUBILITY
- ALTERED INTRA-PROTEIN INTERACTIONS
  - UNFOLDING
  - DENATURATION
  - INCREASED AGGREGATION

IMPAIRED FUNCTIONALITY

Carbonylation
Loss functionality

Emulsifying
Gelling
WHC
Juiciness

Xiong & Decker, 1995
Xiong, 2000
Bertram et al., 2007
Estévez et al., 2011
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

PROTEIN FUNCTIONALITY (WHC)  ↔  PROTEIN CARBONYLATION  ↔  CROSSLINKS

CARBONYLATION

LOSS OF WHC

TYPE OF MUSCLE
PREMINCING
PACKAGING

FREEZE STORAGE

AAS

9 bound water/100g lean meat

CROSSLINKS

SHRINKAGE OF MYOFIBRIL
MECHANICAL CONSTRUCTION MUSCLE

LOSS WHC

PROTEIN CARBONYLATION AND WATER-HOLDING CAPACITY OF PORK
SUBJECTED TO FROZEN STORAGE: EFFECT OF MUSCLE TYPE,
PREMINCING, AND PACKAGING

Mario Estévez,*,† Sonia Ventanas,† Marina Heinonen,† and Zero Puchair‡

Journal of Agricultural and Food Chemistry

AAS

Week 1
Week 4
Week 8
Week 12

FREEZE STORAGE

AAS

Area Units

20

15

10

5

Week 1
Week 4
Week 8
Week 12

FREEZE STORAGE
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

- SENSORY PROPERTIES
  - PROTEIN OXIDATION
  - FAILED TENDERIZATION PROCESS
  - TENDERNESS

**THEORY 1**
Huff-Lonergan et al. 2010
Carlin et al. 2006
Rowe et al. 2004

- DECREASED PROTEOLYSIS DURING AGING
- INACTIVATION OF PROTEOLYTIC ENZYMES

**THEORY 2**
Lund et al. 2007

- FORMATION OF CROSSLINKS
- STRENGTHEN MYOFIBRILS
- TOUGHEN MUSCLE TISSUE

**DNPH ↔ WB SHEAR FORCE**

**ROLE OF PROTEIN CARBONYLS**
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

**SENSORY PROPERTIES**

**PROTEIN CARBONYLATION** → **FLAVOR**

**PROTEIN CARBONYLS** ↔ **FREE AMINO ACIDS**

**STRECKER ALDEHYDES**

- LARGE AMOUNTS OF PROTEIN CARBONYLS
- LARGE AMOUNT OF FREE AMINO ACIDS
- STRECKER ALDEHYDES: MARKERS OF RIPENING
- STRECKER ALDEHYDES: ODOR ACTIVE COMPOUNDS

*Armenteros et al. 2010*
*Córdoba et al. 1994*
*Ruiz et al. 2000*
*Carrapiso et al. 2002*
3/ IMPACT OF PROTEIN CARBONYLATION ON MEAT QUALITY

**NUTRITIONAL VALUE**

- **Protein Carbonyls** → Loss of Essential Amino Acids (Lysine / Arginine)
- **Carbonylated Meat Proteins** → Less Sensitive to Proteolysis → Impaired Digestibility

**DNPH ↔ TRYSIN**

- Morzel et al. 2006
- Santé-Lhoutellier et al. 2007

- Lys / Arg Carbonylation → AAS / GGS
4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

ANTIOXIDANT STRATEGIES

LIPID OXIDATION ↔ PROTEIN OXIDATION

UNPREDICTABLE OUTCOME

- ANTIOXIDANT
- NO EFFECT
- PRO-OXIDANT
4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

**ANTIOXIDANT STRATEGIES**

**NUTRITIONAL STRATEGIES**

**MODIFICATION OF FATTY ACID PROFILE**
- Inconsistent results

**SUPPLEMENTATION WITH TOCOPHEROL**
- Higher protection against carbonylation

**FEEDING ON NATURAL RESOURCES**
- Inhibit protein carbonylation in meat and meat products

Ventanas et al., (2007)
4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

ANTIOXIDANT STRATEGIES

TECHNOLOGICAL STRATEGIES

PACKAGING STRATEGY

80% OXYGEN ATMOSPHERE

VACUUM PACKAGING

Lindahl et al., (2010)
Filgueras et al., (2010)
Zakrys-Waliwander et al., (2010)
## 4/ Antioxidant Strategies Against Protein Carbonylation

### Antioxidant Strategies

#### Ingredients with Antioxidant Potential

<table>
<thead>
<tr>
<th>Authors</th>
<th>Meat Product</th>
<th>Natural Antioxidant</th>
<th>Lipid Oxidation</th>
<th>Protein Oxidation</th>
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<td><img src="antioxidant.png" alt="Antioxidant" /></td>
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<td>Sun et al., (2010)</td>
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<td>PROOX</td>
</tr>
</tbody>
</table>

#### Technological Strategies
4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

ANTIOXIDANT STRATEGIES

PHENOLICS- PROTEINS INTERACTIONS

ACTIOXIDANT MECHANISMS

1. Radical Scavenging Activity
2. Metal Chelating Activity

LYSINE

Fenton Reaction

Fe^{2+} + H_2O_2 = OH^- + OH^-

CATECHIN

AAS
4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

ANTIOXIDANT STRATEGIES

PHENOLICS- PROTEINS INTERACTIONS

CHLOROGENIC ACID

Iron

O2

H2O

QUINONE

IMINOQUINONE

QUINONE

IMINOPHENOL

Lysine side chain

AMINOPHENOL

AMINE-OXIDASE ACTIVITY

Akagawa et al., 2005 (J. Agric. Food Chem.)
ANTIOXIDANT STRATEGIES

WHEN PHENOLIC COMPOUNDS ARE ADDED TO A MEAT PRODUCT...

ANTIOXIDANT ACTIONS  VS.  PRO-OXIDANT ACTIONS

HYDROXYL FORM

\[
\begin{align*}
R_1 & \quad \text{OH} \quad \text{OH} \\
\text{HYDROXYL FORM} & \quad \text{QUINONE FORM} \\
R_1 & \quad \text{OH} \quad \text{OH} \\
\end{align*}
\]

OVERALL ANTIOXIDANT EFFECT

OVERALL PRO-OXIDANT EFFECT
4/ ANTIOXIDANT STRATEGIES AGAINST PROTEIN CARBONYLATION

ANTIOXIDANT STRATEGIES

WHEN PHENOLIC COMPOUNDS ARE ADDED TO A MEAT PRODUCT...

ANTIOXIDANT ACTIONS VS. PRO-OXIDANT ACTIONS

HYDROXYL FORM

PRO-OXIDANT FORM

PHENOLIC COMPOUNDS ARE NOT "ANTIOXIDANT COMPOUNDS" BUT REDOX-ACTIVE COMPOUNDS

CONSISTENT STRATEGIES AGAINST PROTEIN OXIDATION USING PLANT PHENOLICS
5/ FINAL REMARKS

FUTURE CHALLENGES

CHEMISTRY ISSUES

Fate of particular amino acids during processing/storage of meat and meat products
Interaction mechanisms between plant phenolics and myofibrillar proteins

TECHNOLOGICAL ISSUES

Investigate intrinsic (muscle) and extrinsic (technology) factors on the onset of protein oxidation
Managing protein oxidation in the meat industry for the production of better muscle foods

HEALTH CONCERNS

Impact of the intake of oxidized proteins on human’s health
“Ramón y Cajal contract (RYC-2009-03901)”
Research Project: (AGL2010-15134) “Protein oxidation in frozen meat and dry-cured products: mechanisms, consequences and development of antioxidant strategies”