Lipid Oxidation in Muscle Foods
Unique Challenges with Oxidation in Muscle Foods

- Rancidity is a major shelf-life limiting factor in frozen muscle foods
- NaCl generally accelerates oxidation
- Oxidation accelerates rapidly in muscle foods that are thermally processed
- Oxidation in muscle foods can also impact color and protein functional properties
Muscle Food Prooxidants

OXIDATION SUBSTRATES

Lipids

- Oxidation of lipids in muscle initially occurs in the cell membranes
Oxidation of different lipid fractions during storage of herring

Decker and Hultin, 1992
Muscle Food Prooxidants

OXIDATION SUBSTRATES

Lipids

- Oxidation of lipids in muscle initially occurs in the cell membranes
- Oxidation rates are not highly dependent on lipid concentrations
Impact of fat content on lipid oxidation in cooked chicken thigh patties (Ang and Young, 1992)
Muscle Food Prooxidants

OXIDATION SUBSTRATES

Lipids

♦ Oxidation of lipids in muscle initially occurs in the cell membranes
♦ Oxidation rates are not highly dependent on lipid concentrations
♦ Oxidation rates are dependent on fatty acid composition with rates increasing with increasing level of unsaturation
Differences in Lipid Substrates among Species

- Level of unsaturated fatty acids and oxidative stability of raw muscle foods are in the order of:
  
fish > turkey > chicken > pork > beef > lamb

This order does not hold true in cooked muscle foods where iron concentrations are also an important factor.
Muscle Food Prooxidants

OXIDATION SUBSTRATES

♦ Lipids

- Fatty acid composition can be altered by diet in non-ruminants
  - Alters oxidative stability and texture

- Difficult to change fatty acid composition in ruminants due to biohydrogenation
Muscle Food Prooxidants

OXIDATION SUBSTRATES

OXYGEN

♦ Reduction of oxygen with processing equipment and packaging technologies
  • Vacuum
  • Modified atmosphere
  • Oxygen scavengers

♦ There was no difference in TBARS observed in beef stored in MAP at 10 and 20% oxygen.

♦ Must remove oxygen quickly in products which are oxidizing rapidly (e.g. cooked products)
Lipid oxidation in cooked, vacuum-packaged turkey

Ahn et al, 1992
Muscle Food Prooxidants

Iron and copper

- Iron more prevalent than copper
- Iron and copper are bound to proteins to control reactivity (etc, ferritin, transferrin)
- Concentrations are dependent on species and muscle type
Muscle Food Prooxidants

Iron

<table>
<thead>
<tr>
<th>Meat Type</th>
<th>mg iron/100 muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>0.08</td>
</tr>
<tr>
<td>Pork</td>
<td>0.02</td>
</tr>
<tr>
<td>Chicken Light</td>
<td>0.02</td>
</tr>
<tr>
<td>Chicken Dark</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Muscle Food Prooxidants

IRON

♦ Iron distribution is altered by heat processing which in turn can increase lipid oxidation

♦ Minimize iron contamination from:
  • processing equipment
  • water
  • ingredients
Muscle Food Prooxidants

- Hemoglobin and Myoglobin
  - Proteins with iron in hemin moiety
  - Biological role as oxygen carrier and storage
  - Hb = tetramer; MB = monomer
  - Important to meat color
Heme Protein Concentrations in Muscle Foods

- Beef
- Pork
- Chicken Dark
Muscle Food Prooxidants

♦ Myoglobin and Hemoglobin
  • Can promote oxidation by:
    – Ferryl radicals – reaction with hydroperoxides
    – Direct decomposition of hydroperoxides
    – Source of iron and hemin
    – Source of reactive oxygen species

♦ Hemoglobin generally more important in fish than poultry and live stock
Muscle Food Prooxidants

♦ Hydroperoxide Decomposition

- DeoxyHb (purple) is the most prooxidative
  - Protein configuration makes hemin accessible
- OxyHb (red) is a weak prooxidant because bound oxygen alters the configuration of the protein
  - Is still active due to reduced state of iron
- MetHb less reactive because iron is oxidized
Prooxidant activity of HB is dependent on oxygen affinity

- Hb mutants with high oxygen affinity are weaker prooxidants (Richards et al., 2009)
- Fish Hb has weaker oxygen affinity and is more prooxidative
- Decreasing pH decreases oxygen affinity and increased prooxidant activity
Muscle Food Prooxidants

- Hemin is a lipid soluble form of iron that promotes hydroperoxide decomposition
- Hemin release from Hb and Mb is a function of its affinity to the protein
- Hemin affinity is dependent on species (fish weakest) and pH (decrease with decreasing pH)
Muscle Food Prooxidants

- Hb and Mb as source of reactive oxygen species
  - Photosensitizers = Singlet oxygen

Triplet oxygen, $^3\text{O}_2$

Singlet oxygen, $^1\text{O}_2$

Singlet oxygen is highly electrophilic and reacts with electron rich compounds
Photosensitizer generated Singlet Oxygen

Photosensitizer (grd) → Light → O₂ → ¹O₂ → Photosensitizer (ext.)
Muscle Food Prooxidants

♦ Hb and Mb as source of reactive oxygen species
  • Photosensitizers = Singlet oxygen
  • Superoxide Anion generation

\[ \text{Mb- Fe}^{+2} \cdot \text{O}_2 \rightarrow \text{Mb-Fe}^{+3} + \text{O}_2^- \]

OxyMb MetMb
Prooxidants
Heme Proteins

Control

• Remove blood
  – Bleeding
  – Rinsing
  – Red meat and poultry have approx. 20% blood retention

• Avoid bone marrow
Antioxidants General Strategies

- Antioxidant protection in muscle foods can be increased by:
  - Protecting endogenous antioxidants
  - Increasing endogenous antioxidants through dietary supplementation
  - Utilization of antioxidant additives
Muscle Food Antioxidants

- Free radical scavenging antioxidants will become inactivated or lost as a result of:
  - Storage
    - Significant loss in antioxidants can occur prior to the appearance of lipid oxidation markers
  - Processing (heat and salt)
Dietary Antioxidants
Increasing Antioxidant Protection in Muscle Foods

♦ Increasing Antioxidant Concentrations by Dietary Intervention
  • \(\alpha\)-Tocopherol
  • Ascorbate
  • Ubiquinone
  • Glutathione
  • Herb Phenolics
    – Rosemary and oregano extracts can be effective
Increasing Antioxidant Protection in Muscle Foods

♦ Of the dietary antioxidant strategies, why is only α-tocopherol the most effective

• α-Tocopherol has high bioavailability
• α-Tocopherol is incorporated into cell membranes
• α-Tocopherol is retained by tissues
• α-Tocopherol can protect both flavor and color
Ability of Dietary Tocopherol to Inhibit Lipid Oxidation in Cooked Turkey Patties made from Breast Muscle

Wen et al., 1996
Antioxidant Additives

- Synthetic Phenolics
- Tocopherols
- Plant Phenolics
  - Rosemary, Grape seed extract, Green tea
- Nitrite
- Ascorbate and Erythorbate
- Chelators
CHALLENGES OF INCREASING OXIDATIVE STABILITY WITH EXOGENOUS ANTI-OXIDANTS

♦ Antioxidant must be present in oxidizing lipid or at catalyst site
♦ Antioxidant must be stable to inherent muscle enzymes and typical processing conditions
♦ Incorporating antioxidant into muscle difficult in intact meat cuts
♦ Antioxidant must not alter typical flavor and color
Muscle Food Antioxidants

♦ Exogenous Antioxidant Additives
  • Tocopherols
## INHIBITION OF LIPID OXIDATION IN MUSCLE FOODS BY TOCOPHEROLS

<table>
<thead>
<tr>
<th>Tocopherol Type</th>
<th>Muscle Source</th>
<th>Storage Conditions</th>
<th>TBARS Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>Salted Pork (0.04%)</td>
<td>28 d @ -15 C</td>
<td>12%</td>
<td>Decker and Crum, 1993</td>
</tr>
<tr>
<td></td>
<td>Cooked Beef (0.02%)</td>
<td>2 d @ 4 C</td>
<td>0%</td>
<td>St. Angelo et al., 1988</td>
</tr>
<tr>
<td>Mixed Toc. Isomers</td>
<td>Cooked Turkey (0.014%)</td>
<td>3 d @ 5 C</td>
<td>39%</td>
<td>Bruun-Jensen et al., 1994</td>
</tr>
<tr>
<td></td>
<td>Salted Pork (0.035%)</td>
<td>28 d @ -15 C</td>
<td>36%</td>
<td>Decker et al., 1993</td>
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<tr>
<td>Dietary α-Tocopherol</td>
<td>Raw Beef (0.00044%)</td>
<td>45 d @ -18 C</td>
<td>84%</td>
<td>Faustman et al., 1989</td>
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<tr>
<td></td>
<td>Cooked Pork (0.007%)</td>
<td>2 d @ 4 C</td>
<td>50%</td>
<td>Monahan et al., 1992</td>
</tr>
</tbody>
</table>
Muscle Food Antioxidants

♦ Exogenous Antioxidant Additives

• Plant Phenolics
  – Several plant phenolics have been found to inhibit lipid oxidation in muscle foods
  – Rosemary phenolics are the most common phenolics used in muscle foods
  – Flavors associated with plant extracts can sometimes limit use in muscle foods
Muscle Food Antioxidants

♦ Exogenous Antioxidant Additives
  • Dried Plum
    • 3-6% puree had similar activity in cooked pork sausages to a BHA/BHT treatment (Nunez de Gonzalez et al., 2008)
  • Plum juice was effective when injected (2.5 and 5% of brine%) into beef prior to roasting (Nunez de Gonzalez et al., 2008)
Muscle Food Antioxidants

♦ Exogenous Antioxidant Additives

• Ascorbate and Eythrobate
  – Inhibits lipid oxidation by acting as a strong hydrogen donor
  – Can be prooxidative by catalyzing the reduction of iron
  – Prooxidative activity can be minimized by using in combination with chelators
Muscle Food Antioxidants

♦ Exogenous Antioxidant Additives

• Nitrites:
  • Proposed antioxidant mechanisms of nitrites include:
    – Reduction of the catalytic activity of heme-containing proteins
    – Chelation of iron
    – Formation of nitrosated amines which possess antioxidant activity
Muscle Food Antioxidants

♦ Celery

- Contains high amount of nitrate
- Used to naturally cure meats
- Nitrate will inhibit lipid oxidation by inactivating myoglobin and chelating metals
Muscle Food Antioxidants

♦ Grape Seed Extract
  • Contain Antioxidative Procyanidins
  • Are GRAS
  • Limited to 0.05-0.08 % due to astringency problems
  • May be effective as dietary antioxidant
  • Very little work published in meat systems
Muscle Food Antioxidants

- Exogenous Antioxidants are most effective when at the site of oxidation (phospholipids)
- Antioxidant location is influenced by carrier solvent

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Membrane</th>
<th>TAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>56%</td>
<td>44%</td>
</tr>
<tr>
<td>Oil</td>
<td>28%</td>
<td>72%</td>
</tr>
</tbody>
</table>

δ-Tocopherol distribution in haddock as a function of carrier solvent.

Raghavan and Hultin, 2004
Muscle Food Antioxidants

Effectiveness of BHA as a function of carrier solvent.

Raghavan and Hultin, 2005
Muscle Food Antioxidants

♦ Exogenous Antioxidants

• Phosphates
  – Polyphosphates are more effective chelators and antioxidants than monophosphates
  – Polyphosphates can be degraded in raw muscle by phosphatases
  – Polyphosphates are more effective in cooked muscle foods due to increased stability and increased free iron concentrations
## INHIBITION OF LIPID OXIDATION IN MUSCLE FOODS BY PHOSPHATES

<table>
<thead>
<tr>
<th>Phosphate type</th>
<th>Muscle Source</th>
<th>Storage Conditions</th>
<th>TBARS Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monophosphate</td>
<td>Cooked Beef (0.5%)</td>
<td>2 d @ 4 C</td>
<td>28%</td>
<td>Trout and Dale, 1990</td>
</tr>
<tr>
<td>Diphosphate</td>
<td>Cooked Beef (0.5%)</td>
<td>2 d @ 4 C</td>
<td>&gt;98%</td>
<td>Trout and Dale, 1990</td>
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<tr>
<td></td>
<td>Raw Beef (0.5%)</td>
<td>28 d @ -15 C</td>
<td>42%</td>
<td>Mikkelsen et al., 1991</td>
</tr>
<tr>
<td>Triphosphate</td>
<td>Cooked, Salted Pork (0.5%)</td>
<td>3 d @ 4 C</td>
<td>86%</td>
<td>Decker and Crum, 1993</td>
</tr>
<tr>
<td></td>
<td>Cooked Beef (0.5%)</td>
<td>2 d @ 2 C</td>
<td>&gt;98%</td>
<td>Trout and Dale, 1990</td>
</tr>
<tr>
<td></td>
<td>Raw, Salted Pork (0.5%)</td>
<td>28 d @ -15 C</td>
<td>Prooxidative</td>
<td>Decker and Crum, 1991</td>
</tr>
<tr>
<td></td>
<td>Raw Turkey</td>
<td>5 d @ 4 C</td>
<td>21%</td>
<td>Calvert and Decker, 1992</td>
</tr>
</tbody>
</table>
Muscle Food Antioxidants

♦ Proteins
- Dairy, soy, blood and oil seed proteins have been shown to be effective
- Inhibit oxidation by free radical scavenging (sulfhydryls and isoflavones) and metal chelation
- Can alter texture attributes
Processing operations which alter the oxidative balance of muscle foods
Processing operations which alter the oxidative balance of muscle foods

- Particle size reduction
  - Mixes prooxidants with lipids
  - Increases oxygen concentrations
Alterations in the oxidative stability of pork by processing

TBARS mg / kg pork

Ground pork mo. at -15 C
9 Salt, ground pork wk at -15 C
Cooked grd pork d at 4 C

Ground pork 9 mo. at -15 C
Salt, ground pork 4 wk at -15 C
Cooked ground pork 4 d at 4 C
Processing operations which alter the oxidative balance of muscle foods

♦ Cooking = Warmed-Over Flavor
Lipid oxidation in ground pork cooked to different internal temperature

TBARS (mg / kg muscle)

Days

0.1
1.0
2.0
3.0

35 C
60 C
70 C
80 C
90 C

Mei et al, 1994
Processing operations which alter the oxidative balance of muscle foods

♦ Dramatic changes in lipid oxidation as a function of temperature suggests that oxidation rates could be decreased by control of cooking parameters
  ♦ Slow cooking causes a greater change in iron distribution than fast cooking
Processing operations which alter the oxidative balance of muscle foods

♦ Cooking (warmed-over flavor)
  • Activation of Myoglobin
    • Partial Denaturation of Myoglobin Exposes Iron and Increases Reactivity
  • Releases protein-bound Fe
Changes in Fe distribution in cooked beef

Han et al, 1993
Processing operations which alter the oxidative balance of muscle foods

- **Cooking (warmed-over flavor)**
  - Releases protein-bound Fe
  - Activation of Myoglobin
    - Partial Denaturation of Myoglobin Exposes Iron and Increases Reactivity
  - Inactivation of Antioxidant Enzymes
Antioxidant Enzymes in Food which Control Oxidation Intermediates

♦ Superoxide Anion
  • Superoxide Dismutase
    \[ 2 \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O} \]

♦ Peroxides
  • Catalase
    \[ 2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O} \]
  • Glutathione Peroxidase
    \[ \text{ROOH} + 2 \text{GSH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O} \]
Impact of Cooking on Antioxidant Enzyme Activity

% Original Enzyme Activity

Temperature (°C)

CAT
GSH-Px
SOD
Muscle Food Prooxidants

♦ Salting
  • Changes distribution and reactivity of Fe
    • Binding of Na to Proteins can Displace Iron and make it Active
    • Iron Associated with Cl could have greater reactivity
  • Alters antioxidant enzyme activity
Inhibition of antioxidant enzyme activity by NaCl

Lee et al, 1997