Food Law Enforcement

Differentiation of fresh vs. thawed meat
Why differentiate between fresh vs. thawed meat?

- **Quality:** fresh meat is more nutritious and can have and an distinguishable taste

- **Money:** Consumer pay more to get worse quality meat

- **Fairness:** towards customers and other firms

- **Law:** wrong labelling is illegal
Legal Need

Federal Act on Foodstuffs and Utility

Art. 1 The aim of this act is:

a. to protect consumers from foodstuffs and utility articles that may present a risk to health;

b. to ensure that foodstuffs are handled hygienically;

c. to protect consumers from deception relating to foodstuffs.

Art. 18 1 All information relation to a foodstuff, and in particular the properties

Advertising for foodstuffs and their presentation and packaging must not mislead the consumer.

3 In particular, information about a foodstuff or the presentation thereof is considered to be misleading if it is liable to deceive the consumer as to the manufacture, composition, properties, method of production, storage life, origin, particular effects or value of the foodstuff.

Foodstuffs Act, FSA, 1992
Legal Need

Verordnung des EDI

Art. 17 Könnte die Unterlassung einer entsprechenden Angabe zu einer Täuschung führen, so muss hingewiesen werden:

a. auf den physikalischen Zustand des Lebensmittels (z.B. pulverförmig, flüssig);

oder

b. auf die besondere technologische Behandlung, die das Lebensmittel erfahren hat (z.B. gefriergetrocknet, konzentriert, pasteurisiert, geräuchert, mit Ozon behandelt).
Consequences of freezing

- Formation of ice crystals:
  - fast freezing: small crystals
  - slow freezing: fewer, bigger crystals

- Damage of the microstructure

- Local increase in salt concentration

- Denatures proteins and damages cell organelles

- Release of the content of the organelles

Meat Science, 80, 2008, 151-158.
Method requirements

- Precise
  - Discrimination fresh and thawed meat
  - Determination of frozen temperature and time

- Simple and cheap
  - Not too many procedures
  - No expensive instrument required

- Application
  - Applicable to different meat type
DNA based techniques-Comet assay

- Freeze-thaw cycle would cause DNA damage
- The shape of the electrophoresed DNA resembles comets under fluorescence microscopy detection
- The intensity of the comet tail relative to the comet head reflects DNA damage

⭐ Can detect the meat stored at temperature 0-12 °C
⭐ May also work without reference samples from the same origin
⭐ Fast, inexpensive, easy

❌ Not fully validated for other muscle type and animal age
❌ quality of protocol and experimental performance is of crucial importance especially during electrophoresis

Nature Protocols 2006,1, 23-29
Spectroscopy

Visible/ near infrared spectroscopy (NIR)

Color, chemical and water distribution changes during meat freezing

- Have the potential in the analysis of ground meat
- Short time of analysis
- Not fully validated for other muscle type and animal age
- Hardly differentiate different freeze-thaw condition
- Need complex data analysis

7 days
(a) 4 °C;
(b) 0 °C;
(c) -3 °C;
(d) -12 °C;
(e) -18 °C.

Average visible/NIR reflectance spectra in the 410–2490 nm

Spectroscopy

Raman spectroscopy

Based on the protein secondary structure modification

With the increase of the storage time, the intensity at 1655 cm\(^{-1}\) decreased

\(\alpha\)-helix decreased, \(\beta\)-sheet increased

Spectroscopy

Raman spectroscopy

- Requires only small sample
- Non-destructive, no homogenization and extraction required, no use of dyes, labels, or other enhancing agents

Need complex data analysis

Meat Sci, 2008, 80, 132–149
Barbara Güntherdt, Jianbo Zhang
Nuclear magnetic resonance (NMR)

NMR spectroscopy
Characterize the water and structure features in meat
Transverse relaxation time significantly decreased after thawed meat

<table>
<thead>
<tr>
<th>t-Test</th>
<th>Condition</th>
<th>pH</th>
<th>Per cent water</th>
<th>$T_1$ (s)</th>
<th>$T_2$ (s)</th>
<th>$T_{1sat}$ (s)</th>
<th>$M_{sat}/M_0$</th>
<th>MT (s)</th>
<th>$M_0$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Fresh vs. thawed</td>
<td>Standard</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
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<tr>
<td>Lamb</td>
<td>Fresh vs. thawed</td>
<td>Standard</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
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<td>$P &lt; 0.01$</td>
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<tr>
<td></td>
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<td>Tenderloin</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
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<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Standard vs. tenderloin</td>
<td>Thawed</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pork</td>
<td>Fresh vs. thawed</td>
<td>Standard</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
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<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

Nuclear magnetic resonance (NMR)

NMR spectroscopy

⭐️ Accuracy
highly successful

❌ Instrument is not easily accessed
NMR is quite expensive!!

❌ Hardly differentiate different freeze-thaw conditions
Bioimaging

Microscopy

Size of ice crystals correlates to freezing condition
Slower the freezing rate, the larger the crystals

- Inexpensive
- Can detect the meat stored at temperature 0-12 °C
- Can detect the meat stored with long term

- Can not differentiate certain storage conditions
- Complex sample preparation-thin cuts and often staining

J. Food Sci. 1988, 53, 1631-1637
Bioimaging

Electron microscopy

Principle: Microstructure deterioration of muscle fiber

★ Greater resolution and magnification. e.g. Z line degradation

★ Can detect the meat stored at temperature 0-12 °C

★ Can detect the meat stored with long term

X Sample preparation-cryofixation, dehydration, embedding, or staining with heavy metal

X Currently no exact relationship between the storage condition and deterioration degree

X Under certain freeze-thaw condition, no obvious structural damage observed

Meat Sci, 2008, 80, 132-149
J. Animal Sci. 2004, 17, 1291-1295
Enzymatic Method (HADH):

**Principle:** Increased amount of enzyme activity in meat press juice

- Application of mechanical force or centrifugation to collect press juice

- Measure the activity of a defined enzyme:
  - E should be released during freezing, but not during storage in the refrigerator
  - Activity of E should be constant
  - E should be easily detectable in the press juice
Enzymatic Method (HADH):

Spectrophotometric method:

\[
\text{Acetoacetyl-CoA + NADH + H}^+ \rightleftharpoons_{\text{HADH}} \beta\text{-Hydroxybutyryl-CoA} + \text{NAD}^+
\]
Enzymatic Method (HADH):

- Colour test method:

(I) \( \text{Acetoacetyl-CoA} + \text{NADH} + \text{H}^+ \xrightleftharpoons{} \text{HADH} \beta\text{-Hydroxybutyryl-CoA} + \text{NAD}^+ \)

(II) \( \text{NADH} + \text{H}^+ + \text{Meldolablau (oxidiert)} \rightarrow \text{NAD}^+ + \text{Meldolablau (reduziert)} \) (blau) (farblos)
Enzymatic Method (HADH):

- Not applicable to ground meat

- Minimal freezing temperature required (-12 °C)

- Applicable to beef, veal, pork, mutton/lamp, game and poultry

→ Method of Choice
SOP – Standard Operating Procedure

- Sampling
  - 400g fresh sold sample
  - divide in 4 portions
  - store 2 portions (fridge)

- Sample preparation
  - 2 remaining portions are centrifuged
  - meat juice is collected

- Dilution
  - phosphate buffer (0.05M, pH 7.6)
### SOP – Standard Operating Procedure

#### Tabelle 1. Angaben zur Durchführung der Methoden zur Unterscheidung zwischen Frischfleisch und aufgetautem Gefrierfleisch

<table>
<thead>
<tr>
<th>Tierart (Muskelart)</th>
<th>Photometrischer Nachweis</th>
<th>Farbstest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Verdünnung des Preßsaftes</td>
<td>Aktivität im Preßsaft (U/ml) des gefrorenen u. aufgetauten Gewebes</td>
</tr>
<tr>
<td>Rind</td>
<td>1:200</td>
<td>&gt; 3,5</td>
</tr>
<tr>
<td>Kalb</td>
<td>1:200</td>
<td>&gt; 3,5</td>
</tr>
<tr>
<td>Schwein</td>
<td>1:200</td>
<td>&gt; 6,0</td>
</tr>
<tr>
<td>Schaf</td>
<td>1:200</td>
<td>&gt; 5,0</td>
</tr>
<tr>
<td>Hase</td>
<td>1:300</td>
<td>&gt; 7,0</td>
</tr>
<tr>
<td>Reh</td>
<td>1:300</td>
<td>&gt; 7,0</td>
</tr>
<tr>
<td>Huhn (Brustmuskultur)</td>
<td>1:200</td>
<td>&gt; 5,0</td>
</tr>
<tr>
<td></td>
<td>(Schenkelmuskultur)</td>
<td></td>
</tr>
<tr>
<td>Ente (Brust- und Schenkelmuskultur)</td>
<td>1:400</td>
<td>&gt; 20,0</td>
</tr>
<tr>
<td>Gans (Brust- und Schenkelmuskultur)</td>
<td>1:400</td>
<td>&gt; 20,0</td>
</tr>
<tr>
<td>Pute (Brustmuskultur)</td>
<td>1:200</td>
<td>&gt; 5,0</td>
</tr>
</tbody>
</table>

Z. Lebensm. Unters. Frosch. 1987, 184, 115-121.
SOP – Standard Operating Procedure

- **Preparation**
  - 2.6 ml Phosphatbuffer
  - 0.2 ml EDTA-Solution (34.4mmol)
  - 0.05 ml NADH-Solution (7.5mmol)
  - 0.1 ml diluted sample

- **Measurement**
  - after equilibration 0.05 ml Acetoacetyl-CoA are added
  - UV/Vis spectra are recorded (340 nm)

- **Evaluation**
  - From the slope of the results (A versus t) the enzyme activity can be calculated
Case with no reliable results:

- Grinding also destroys cells  \[\rightarrow\] false positive

- Shock freezing doesn’t destroys cells

- Temperature not low enough  \[\rightarrow\] false negative

- Method for cooked meat?
Ensure that SOP works:

- Validate the method

- Control experiments:
  - Compare previous frozen meat with fresh one of the same sample
  - Testing of samples in different laboratories
  - Test a sample of a certified lab
Suggestions

- Warn the firm
- Increase the test frequency and meat types
- Announcement to consumers
- Penalty
Thank you for your attention!

Questions