Multimodal and multispectral imaging approaches to study the diffusion of pepsin during in vitro digestion of meat

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Meat Qualities

**Health quality**
- Microbiology
- Contaminants
- New formed products

**Organoleptic quality**
- Colour, Texture
- Jutosity, Tenderness

**Technological quality** (processing properties)
- Cooking yield
- Cutting yield
- Emulsifying properties

**Nutritional quality**
- Composition
- Essential amino acids
- Vitamin
- Fatty acids

**Bioaccessibility of digestive enzymes to the muscle food?**

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In vitro gastric digestion

2 hours, 37°C Solution of pepsin 125U/mg of protein, pH 1.8

Scientific procedure

Cryofixation (-160°C) → Cryosections (6-10 µm depth)

Effect of the digestion juice on meat structure and pepsin detection

<table>
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<th>Tissues/cells</th>
<th>Cells/molecules</th>
<th>Molecules/chemistry</th>
<th>Element</th>
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<td>Optical microscopy</td>
<td>Optical microscopy immunohistochemistry</td>
<td>Microspectroscopies Chemical imaging (IR, UV, Raman, TOF SIMS)</td>
<td>Microspectroscopies Chemical imaging Fluorescence X Microanalyse X ToF-SIMS</td>
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</table>
Pepsin digestion of raw ST muscle

Formation of a clearer « digestive halo » in the sample periphery

pepsin digestion of 60°C heated ST muscle

Swelling of the muscle fibers in the halo. Cumulative effect of low pH and pepsine digestion of extracellular matrix.
Clogging disturbs transfer of digestive solution

Astruc et al. 2012, Food Structure Digestion and health congress, Palmerston North, New Zealand
Pepsin digested raw ST muscle

HES staining

Immunohistofluorescence of laminin

Lost of laminin antigenicity in the halo

⇒ alteration of ECM by the digestion solution
Infrared microspectroscopy on label free section: principle

Macromolecular level

70,000 spectra (2 hours acquisition)
protein
macromolecular structure

reflected by
$1654 \text{ cm}^{-1}$ absorbance assigned to $\alpha$-helices

"Protein unfolding"

*Motoyama et al. (in preparation)"
**Maldi-Tof: Matrix Assisted Laser Desorption Ionization - Time of Flight**

**Principle of time-of-flight analyzer:**

The travel time of the ions in the tube depends only on their mass.

The lighter ions will reach the detector before the heaviest ones.

The measurement of time makes it possible to calculate the m/z and therefore the mass of the ions.
Mass Spectrometry Imaging: MSI

To mass spectrometer (TOF....)

Matrix

Analytes

Tissue section

Glass slide

Matrix

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In situ proteolysis characterization

Cryo-fixed digested meat sample

37°C
2 hours
Solution of pepsin
125 U/mg of protein.

10 µm cryosections

Tissue structure
Protein antigenicity

Histochemistry

MALDI Imaging

Proteines peptides maps

Pepsin digestion

linear mode from m/z 2,000 to 30,000 ; spatial resolution of 100 µm.
Normalization and extraction of spectra from regions of interest using FlexImaging software (3.0, Bruker Daltonics).
Maldi-ToF mapping of a digested sample cryosection

Macromolecular level

Pepsin digestion

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Molecular imaging of proteins

Less m/z 2600 proteins in the digested part.

Molecular imaging of peptides

More m/z 1520 peptides in the digested part.

Molecular imaging of peptides

Co-register image of immuno-labeling of laminin


Boland et al. 2014. Digestion and digestibility of meat: an investigation of the effects of processing and preparation. NZIFST Conference, 1st - 3rd July, Christchurch, New Zealand
Until now, visualization of the effects of digestion juice (pepsin + pH) on meat structure

Is it possible to detect pepsin directly on digested meat sections?
The most useful technique to localize a protein in a biological tissue: immunohistochemistry

- **Anti-pepsin primary antibody**
- **Wash to remove excess of anti-pepsin**
- **Cy3 fluorescent dye - secondary antibody**
- **Wash to remove excess of secondary antibody**
- **Observation Image acquisition**

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bright spots only visible in the halo of digestion

Low pepsin content

initially? extraction in the washing baths?
Labelling of pepsin with a dye which remain stable at pH 2: Bodipy dye

BODIPY® TR-X NHS ester 634 gmol⁻¹

327 aa, 35 kD (Tang et al. 1973)

Pepsin aggregation

Steric hindrance?

Loss of pepsin activity

Pepsin activity?

Biochemical analysis
Label free pepsin detection by ToF-SIMS imaging

Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) provides elemental, chemical state, and molecular information from surfaces of solid materials.

10 µm cryosections

Mass spectroscopy mapping

Science et Surface laboratory, Ecully, France

ToF-SIMS V (ION-TOF GmbH, Munster, Germany)
Protein map

C15H14NO+
MC: 6; TC: 4.543e+004

Triglyceride map

Sum of: C35H67O4+, C37H69O4+, C33H63O4+, C39H71O4+
MC: 14; TC: 1.021e+004

Fatty acid map

C16H31O2-
MC: 10; TC: 5.996e+004
C18H33O2-
MC: 10; TC: 5.582e+004
C18H35O2-
MC: 10; TC: 2.961e+004
INORGANIC SPECIES
High signal for
- Na and Cl
- Negative ions of Nitrogen species CN, CNO, CₓN and CₓNO
- Phosphorus species POₓ (PO₃ most represented)

ORGANIC SPECIES
High signal for
- (CₓHᵧ) aliphatic and aromatic
- Positive ions of nitrogen species CₓHᵧN (C₄H₈N and C₅H₁₂N most represented)
- Oxygenated organic species CₓHᵧOz with the C₃H₃O⁺ species strongly detected

Mass spectroscopy
Pepsin powder

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Conclusions

Methods: Diffusion of pepsin in meat can be assessed by imaging

Indirect methods: Histology, maldi tof imaging, FT-IR microspectroscopy

- allow to characterize some effects of «digestion juice» entrance in the food including effects of pepsin, pH, other hydrolases together.

Direct methods of pepsin detection: immunohistofluorescence, SIMS imaging

- Immunohistofluorescence and pepsin labelling show their limits for pepsin detection: High background for immunohistofluorescence, loss of pepsin activity after the dye have been bind.

- SIMS allows the label free detection of chemicals species specific to pepsin. This approach need confirmation, perhaps by labelling the pepsin with stable isotopes that are easy to evidenced by SIMS.
Conclusions

Mechanisms of gastric digestion of meat:

- Pepsin diffuse in meat through a homogeneous front.
- Pepsin seems also to diffuse along the perimysium sheaths.

Perspectives

Work in progress: Chewing taken into account
Thank you for your attention
Any questions?