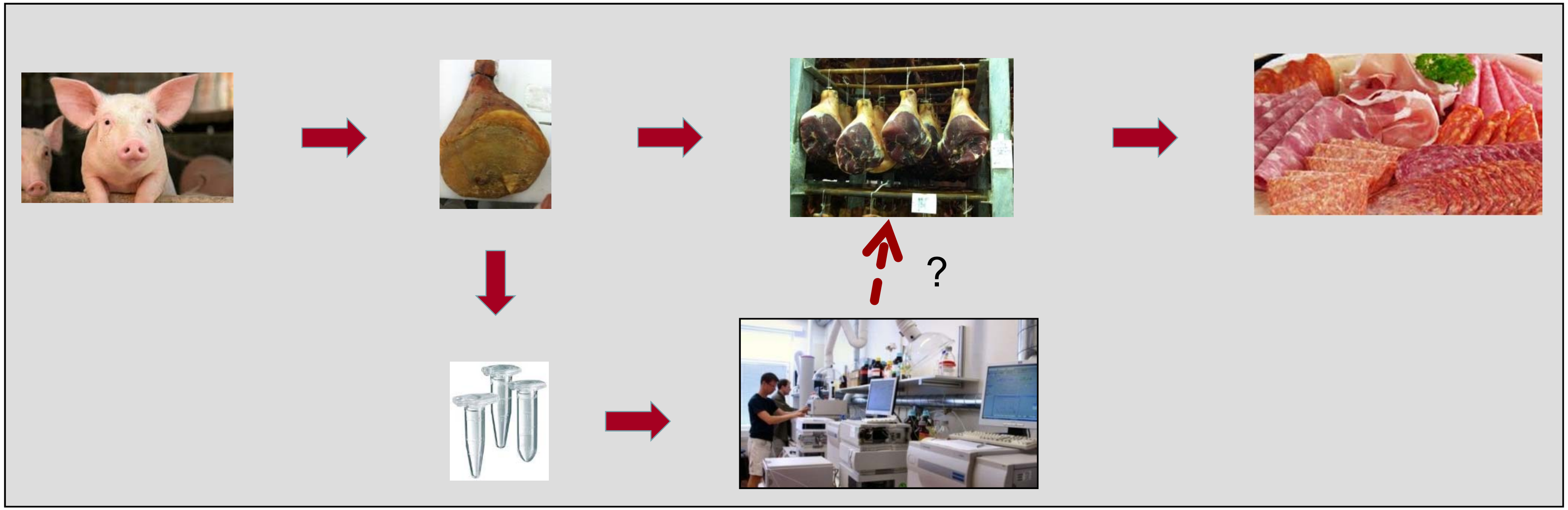
CHARACTERIZATION OF RIPENING PROCESSES BY MASS-SPECTROMETRIC ANALYSES



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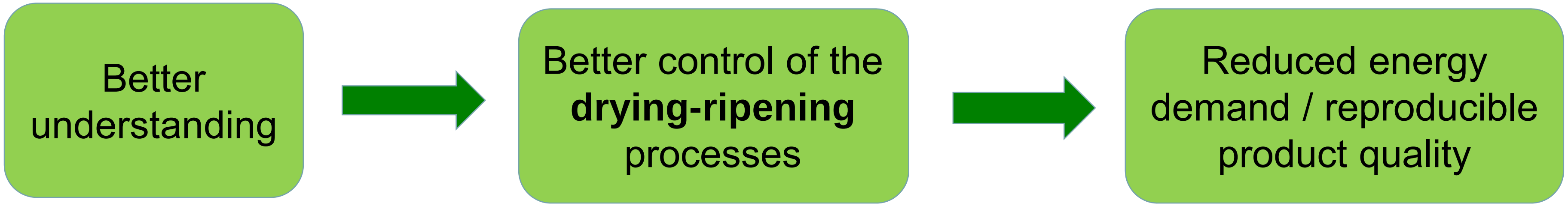
Motivation



Better understanding

Better control of the **drying-ripening** processes

Reduced energy demand / reproducible product quality

- Changes of the drying conditions affect the product properties
- A tool is needed that can describe the effects of changed conditions on the ripening processes

→ Quantitative analyses of compounds (= "marker compounds") that are formed during the ripening process, by use of mass-spectrometry

Methods

- **High-resolution mass spectrometry**
  - LC-MS – ESI – QTOF
    - High mass-accuracy
    - Identification of compounds by use of databases
  - Sample preparation
    - Freeze-dried, homogenized in in water, centrifuged and filtered
  - Un-targeted ("fingerprint") mode:
    - Muscle metabolites
    - Small peptides
    - Free amino acids and amino acid degradation products
    - Other water-soluble compounds

Raw materials and strategies

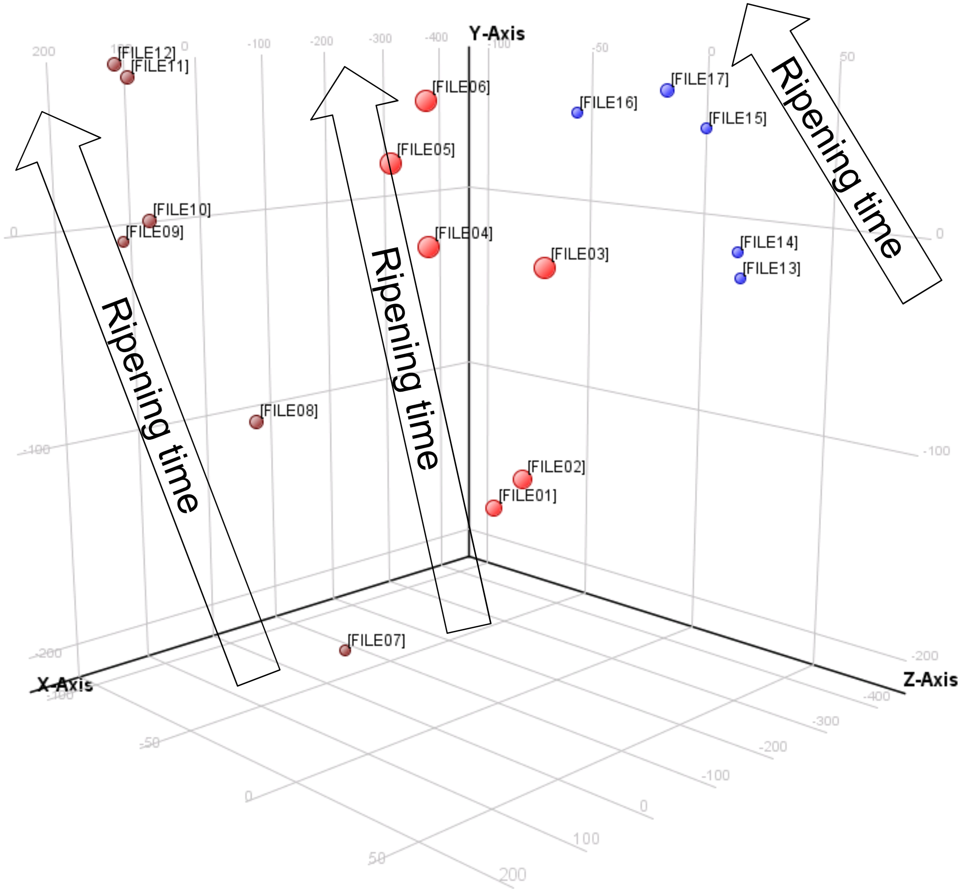
- **Small scale laboratory experiments**
  - Small slices of meat, salted or pre-dried to different water activities (a_w), stored at constant conditions

  ➔ Reaction rates as a function of a_w and temperature

- **Samples from a production facility**
  - Long-time ripened ham (24 months production process)
  - 4 (0) - 10 (6) - 16 (12) - 22 (18) - 24 (20) months processing (drying/ripening)
  - New samples from the same hams after 3 and 6 months

Results

Laboratory experiments

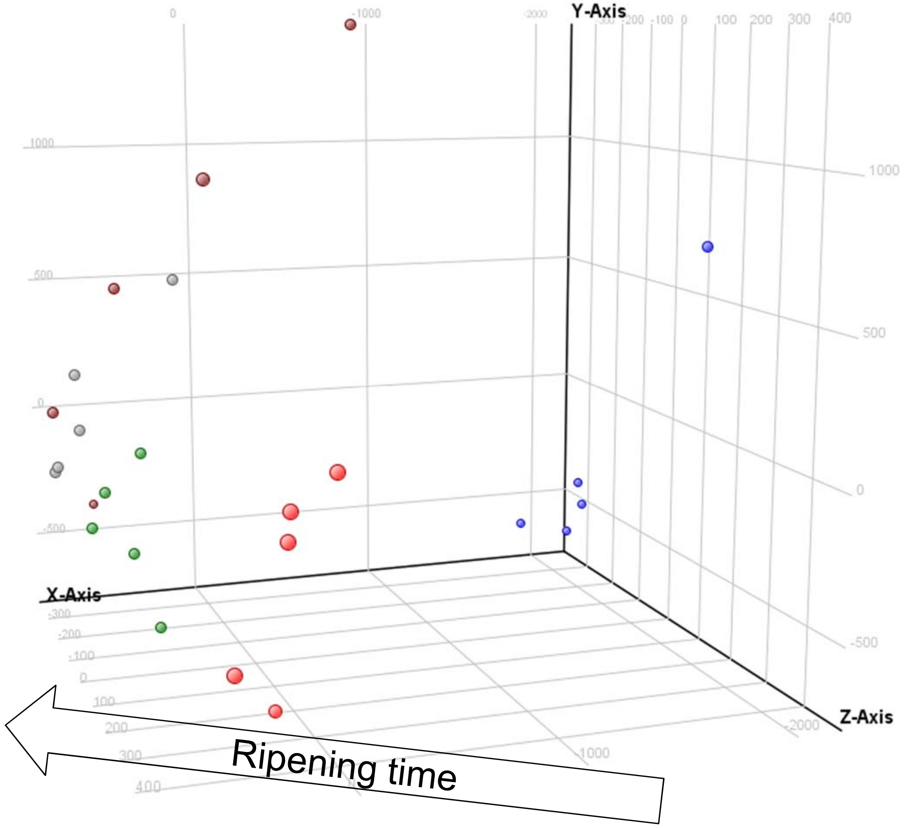


Red: Fresh
Brown: Dried (a_w=0.92)
Blue: Salted (a_w=0.95)

All compounds detected at more than one time-point in all three sample sets

Results

Hams from production facility

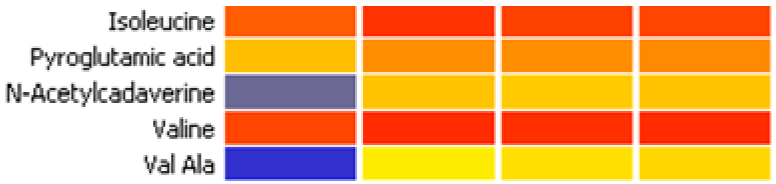


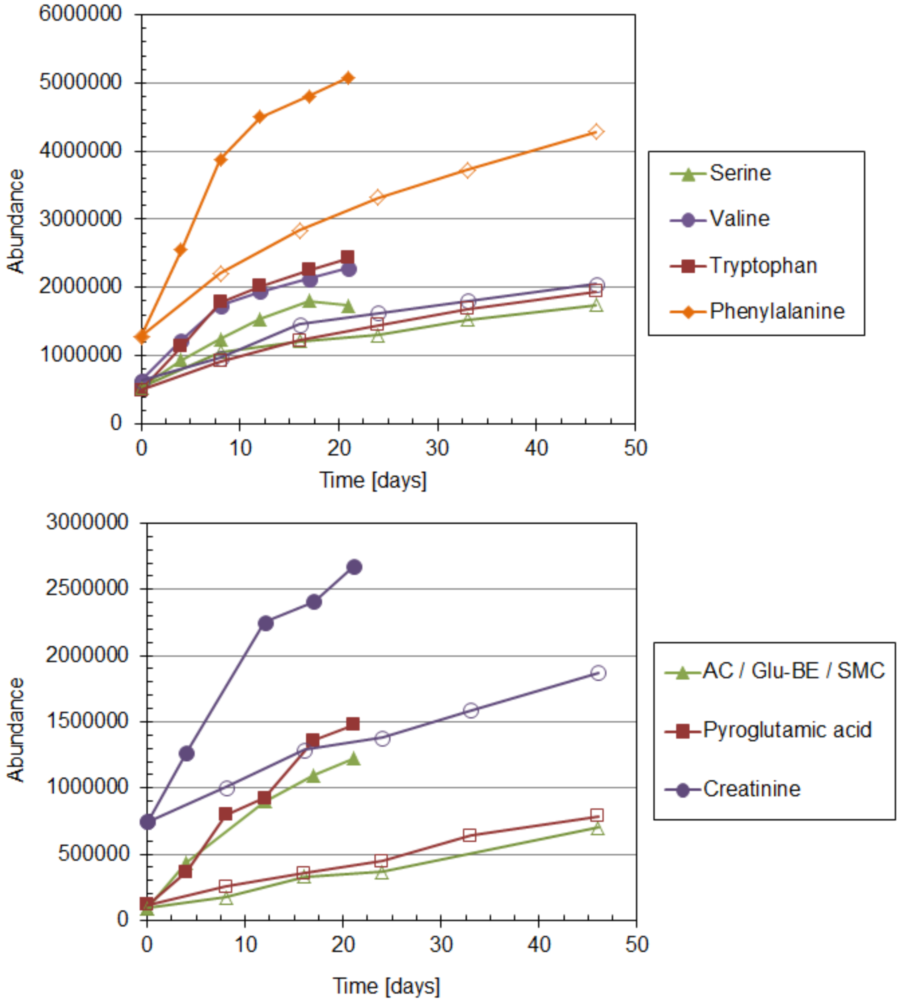
- ● 4 (0) months
- ● 10 (6) - " -
- ● 16 (12) - " -
- ● 22 (18) - " -
- ● 24 (20) - " -

Compounds present in at least 4 of 5 replicates at one time point

Identification of compounds changing with time

Small-scale experiments – Effect of temperature

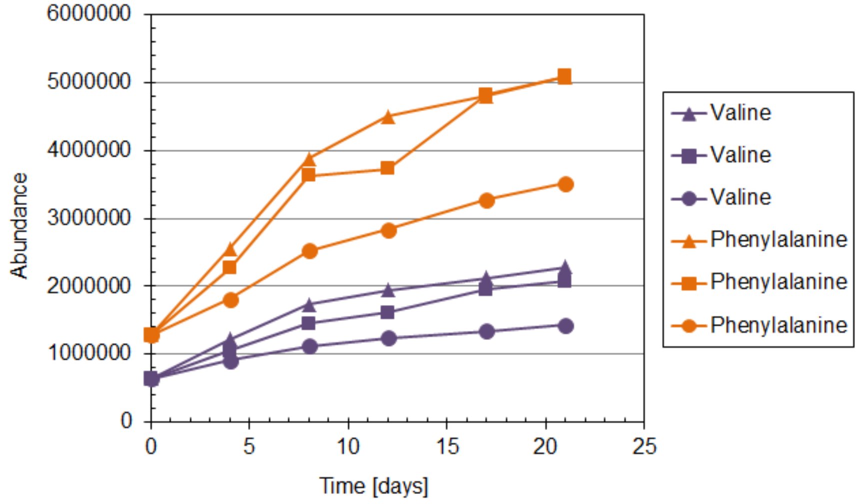


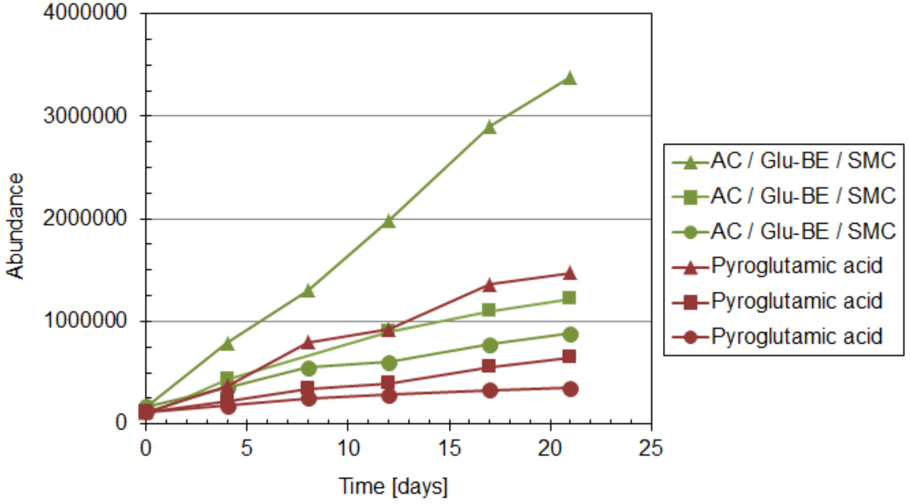


Closed symbols: 25 °C
Open symbols: 13°C

Identification of compounds changing with time

Small-scale experiments – Effect of salt





NaCl added:

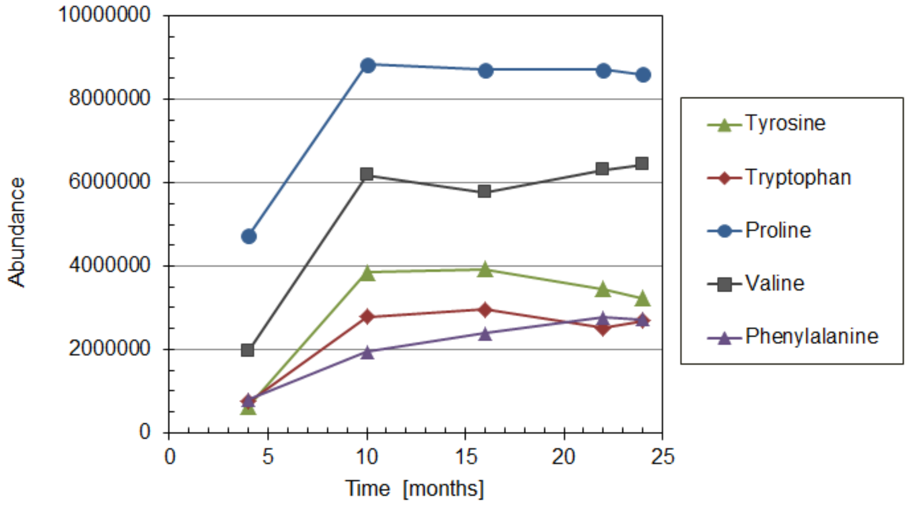
▲: 0

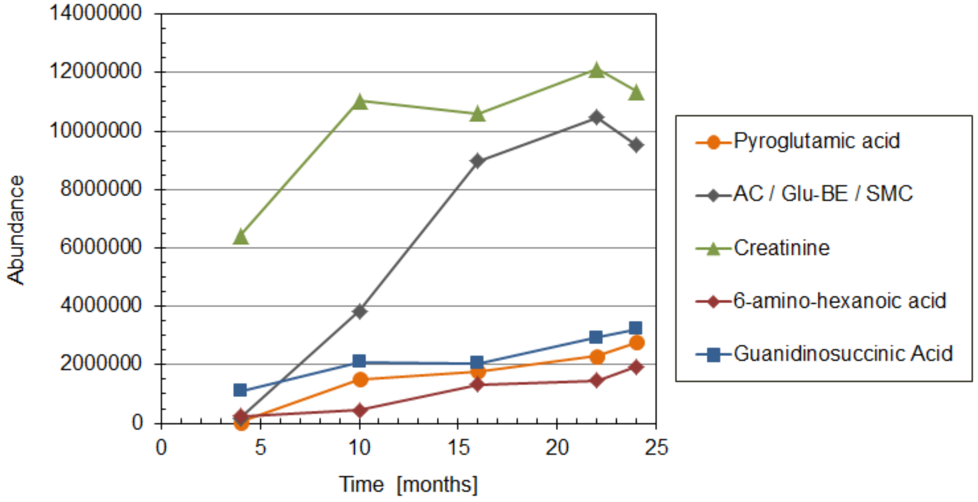
■: 4 % (a_w= 0.96)

●: 8.5 % (a_w=0.92)

Identification of compounds changing with time

Production samples





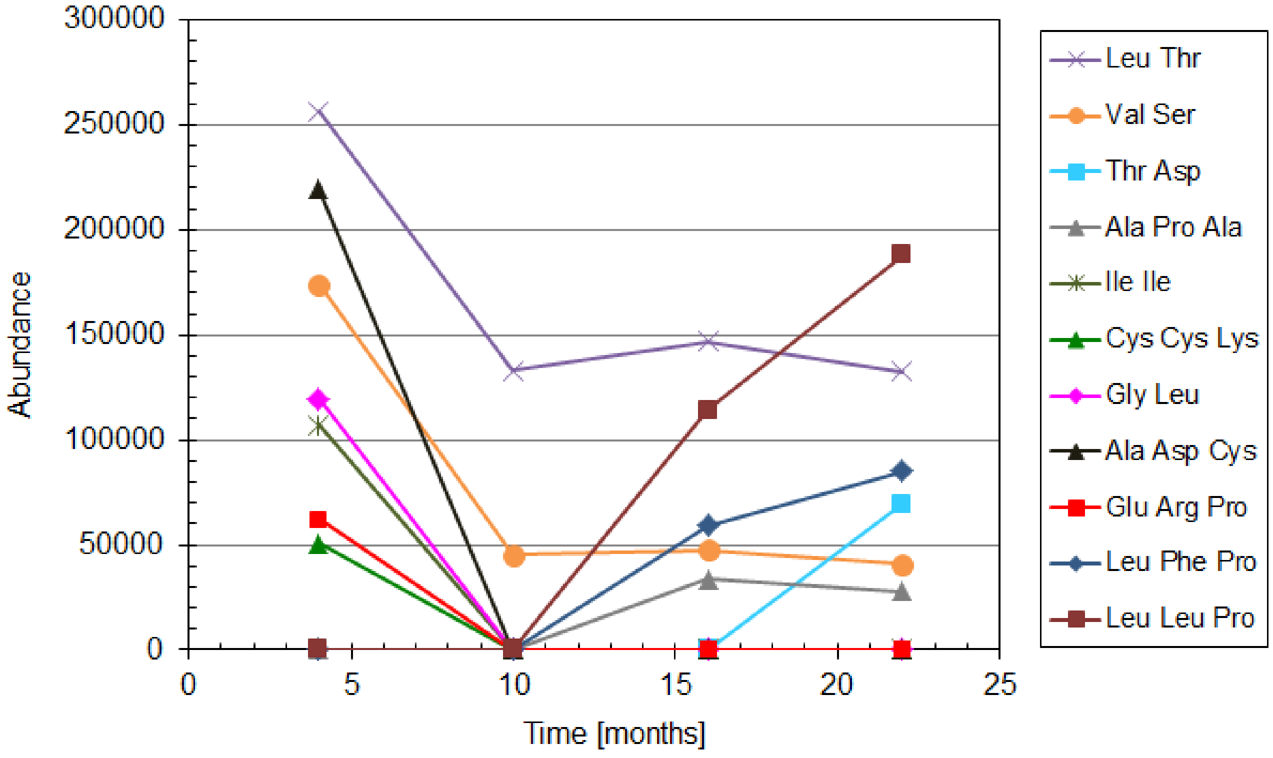
- "Most" happens before 10 months (6 months drying-ripening)

  => A wide range of marker compounds for this period
- For the later stages (>10 (6) months): Amino acid degradation products and some other metabolites

Identification of compounds changing with time

Production samples

Peptides:



Summary and conclusions

- The total "metabolomes" show a clear development with time both in laboratory tests and production samples
- Distinct differences between series with different water activity
- Identified the time course of:
  - Free amino acids

    (increasing the first 7-10 (3-6) months, minimal changes after 10 (6) months)
  - Di- and tripeptides

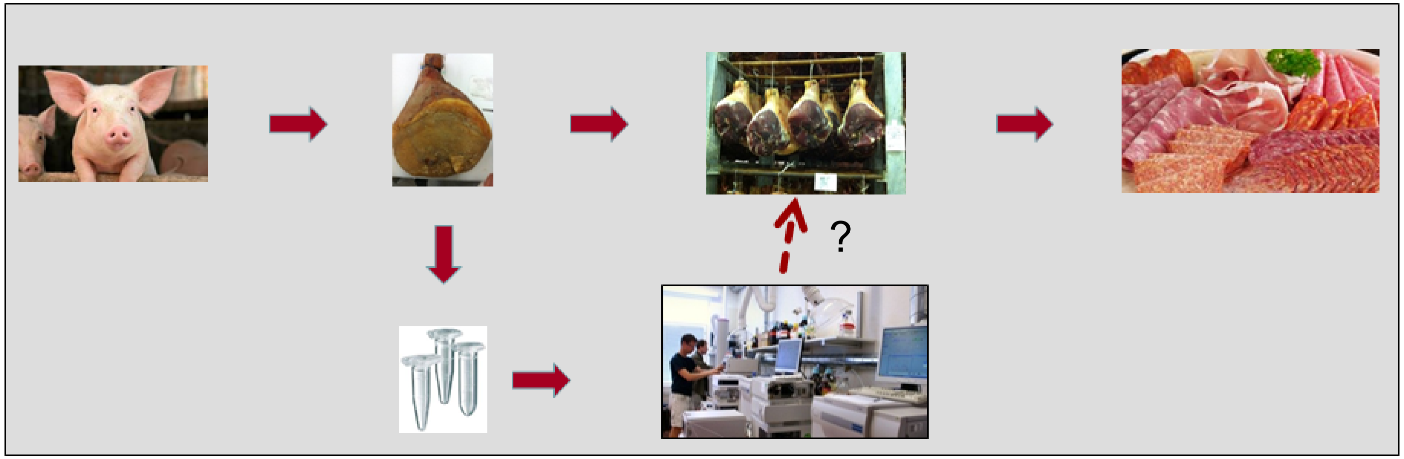
    (most have disappeared after 6 months, a few new are appearing)
  - Some other compounds (ao amino acid derivatives and degradation products)
- A high number of potential marker compounds for the first stages, fewer for the last period

On-going and future work

- A more thorough processing of the data sets to identify relevant compounds with lower abundance
- Select 8-10 potential marker compounds and verify the identity by MS/MS
- Ideally - peptides where the identity of the source protein can be determined, but require MS/MS-analyses
- Use standards and QQQ-MS for quantitative analysis of the selected compounds and develop equations describing their formation rates as a function of a_w
- Verify in:
  - Laboratory scale drying experiments
  - Production samples

Future work

- Integrate in mathematical models for the drying kinetics and simulate the effect of drying conditions on the ripening



People involved

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  - Hanne Haslene-Hox
  - Kristin Reine
  - Inga Marie Aasen
- NORTURA:
  - Per Berg
  - Anne-Mette Grønli

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