

**For how long, and at what temperature, has my  
steak been stored prior to purchase?**



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# Overview

- Analytical methodology.
- Rationale.
- Experimental design.
- Results.
- Conclusion.

# Chemical profiling

- Profiling methods are those that have the power to make a wide range of measurements simultaneously.
- They are usually applied to complex mixtures such as foods.
- Chemical profiling techniques include NMR, FT-IR, Raman spectroscopy and some types of mass spectrometry.
- They provide chemical signatures that can be interpreted to determine the composition of uncharacterised test samples.
- The methods are often used to find statistically significant differences between samples.

# Chemical profiling methods

Non-targeted

FT-IR spectroscopy

NMR spectroscopy

Mass spectrometry

Targeted

Increasing  
specificity

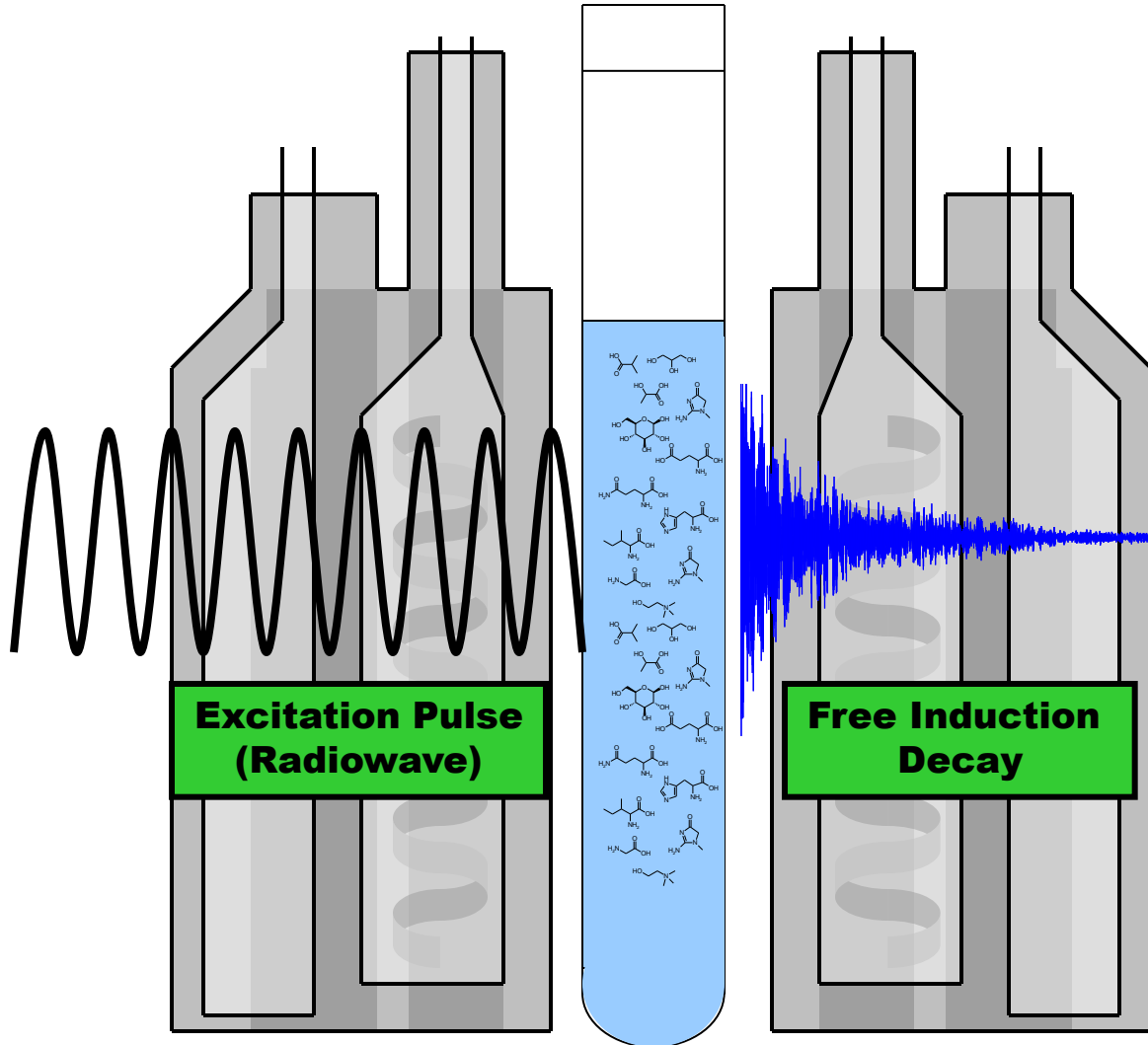
Increasing  
coverage



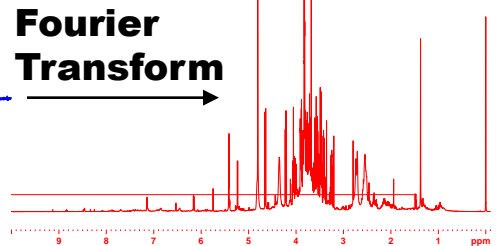
# Nuclear Magnetic Resonance spectroscopy



The Food and Environment  
Research Agency

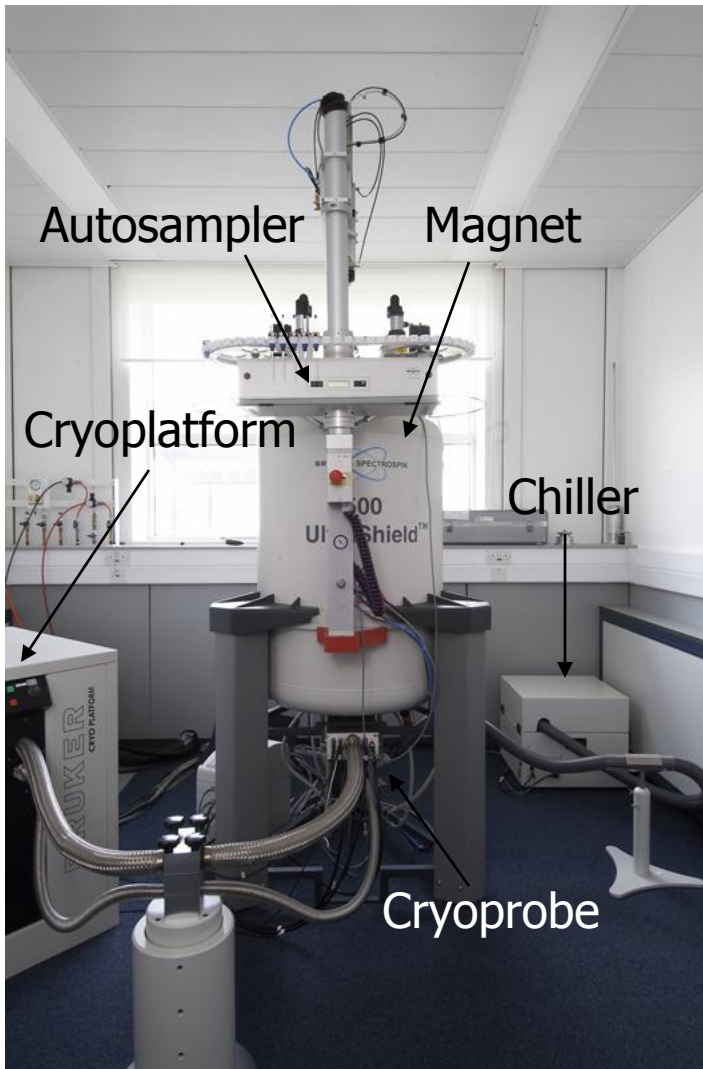


Radiowave causes transitions between energy states and this induces a current that can be detected as the FID.



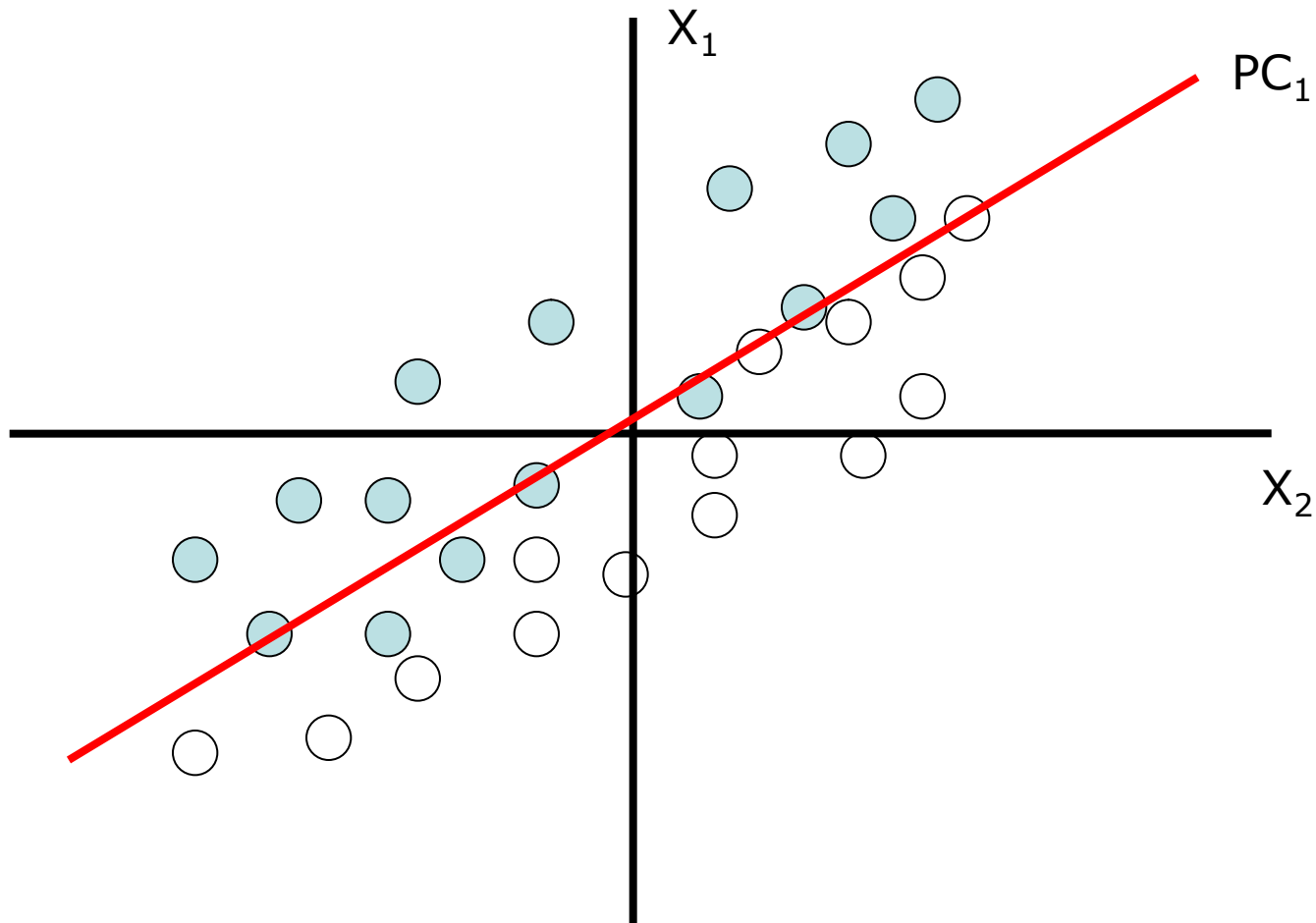
**NMR Spectrum**

# NMR spectroscopy

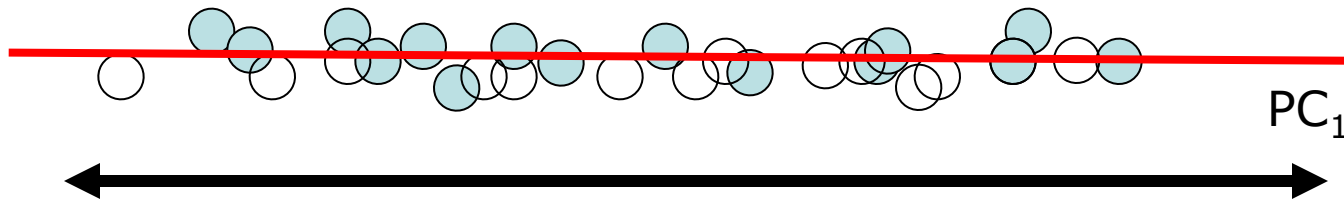


- **High throughput**
- **Unbiased**
- **Unique “virtual” separation**
- **Identification of unknowns**
  - Multinuclear chemical shifts
  - *J*-couplings
  - Peak intensities
  - NOE
  - Diffusion rate

# Principal components analysis (PCA)

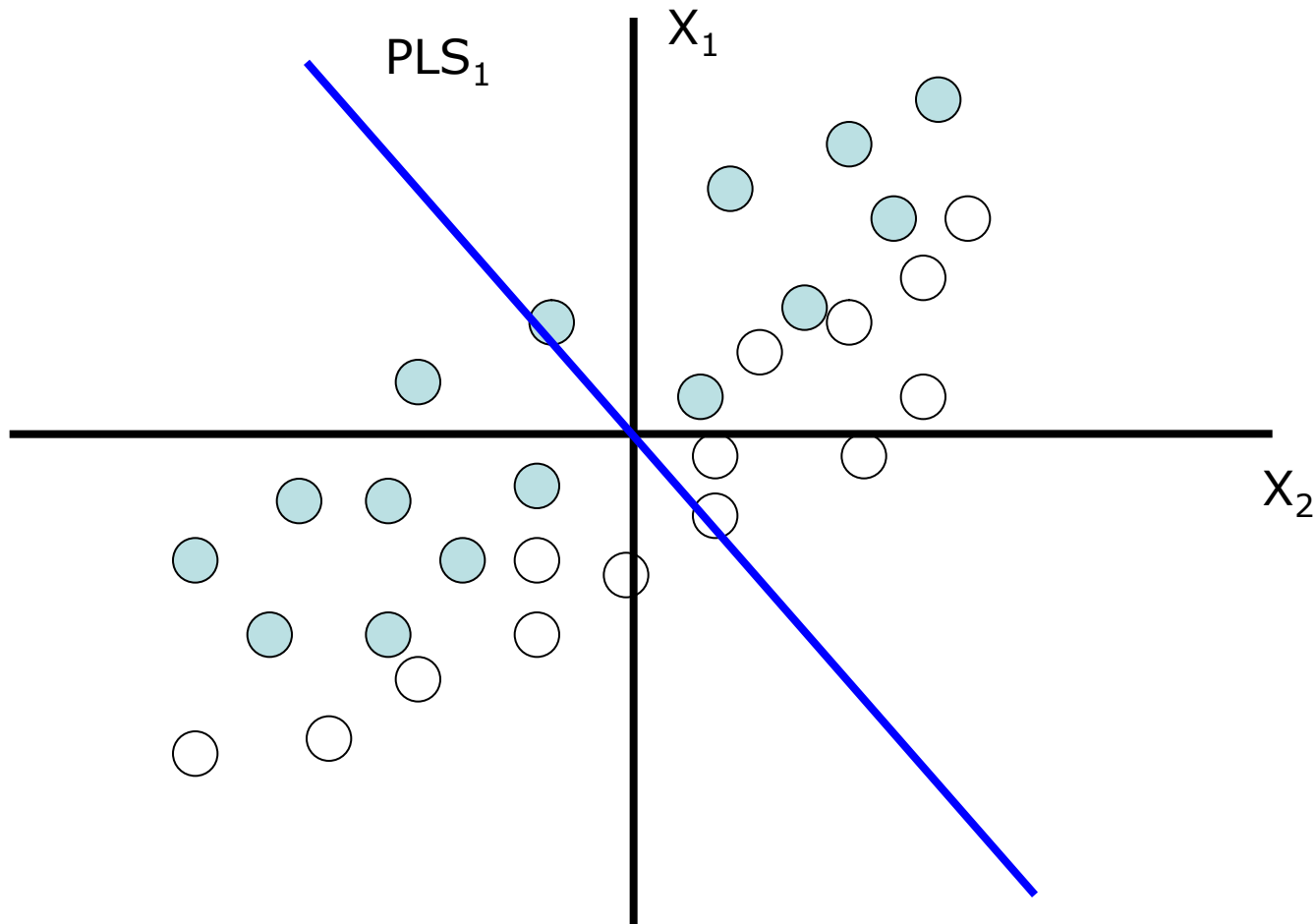


# Principal components analysis (PCA)

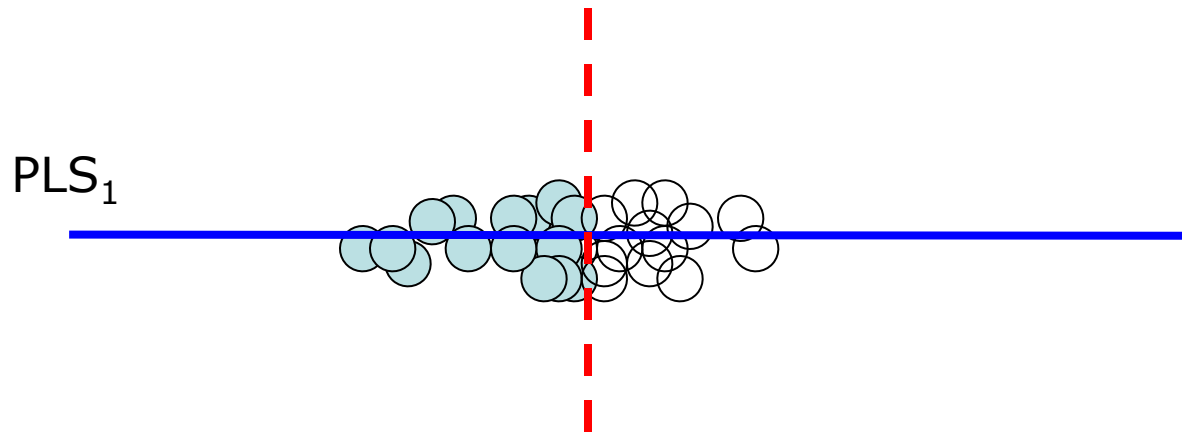


Maximum variance in the data

# Projection to latent structures (PLS)



# Projection to latent structures (PLS)



Maximum discrimination between groups.

# Dry and Wet Aging



- Hung at 1°C
- Weight loss and mould growth = meat loss
- Expensive
- Not now common practice for general meat

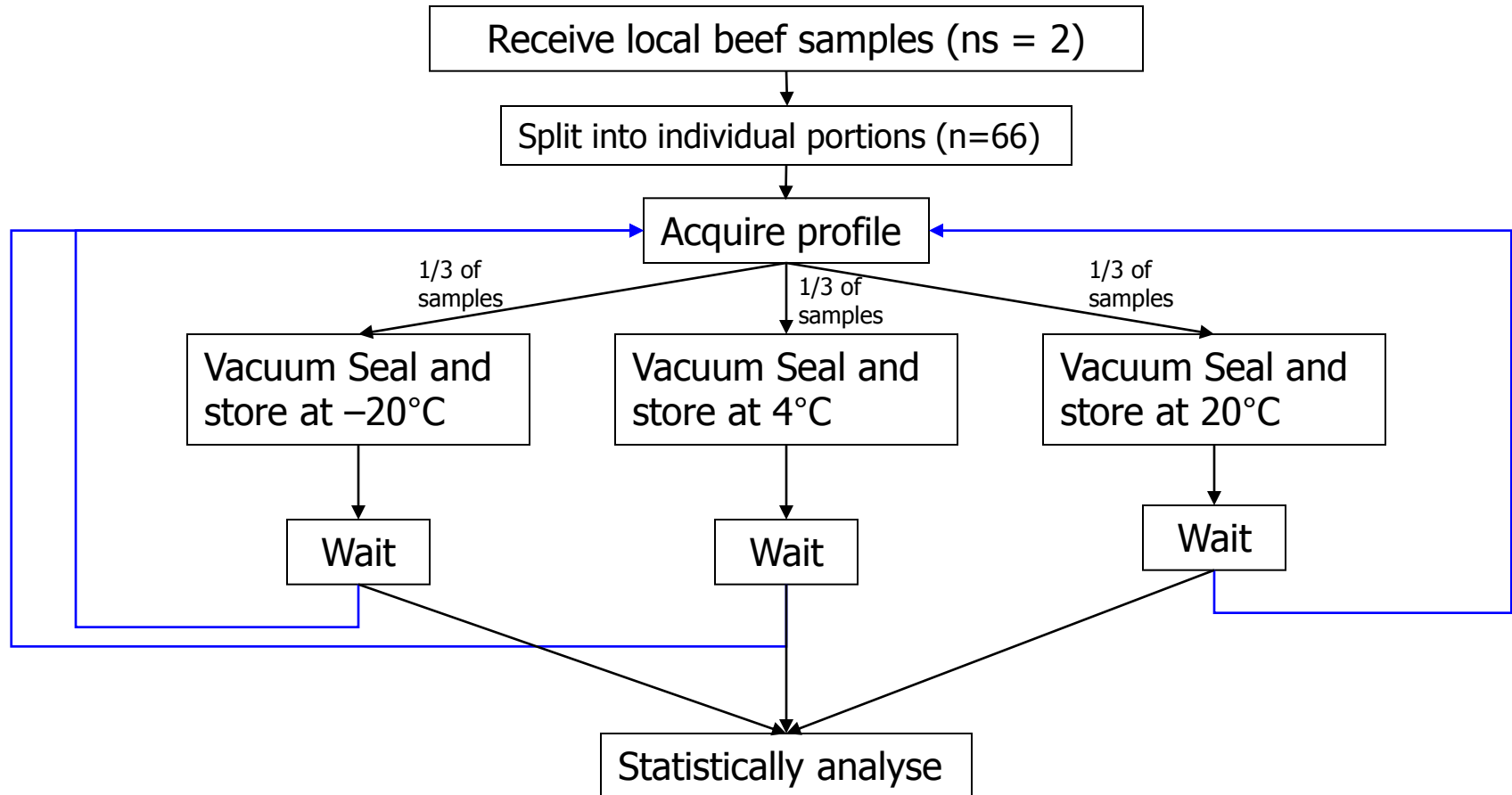
- Vacuum sealed & refrigerated
- No weight loss or mould growth
- Cheap
- Used for supermarket quality meat.

# Meat Aging



- Meat labelled as aged for 21 days
- Used to promote sale of beef
- Can we tell if this is true?

# Experimental design



# Meat aging study

- Striploin (*Longissimus dorsi*) chosen as cut of meat.
  - More uniform in consistency than most cuts.
  - High valued cut of meat therefore fraudulent practices most likely.
  - Large enough to provide enough meat for study.
- Analysis performed daily throughout the week for 28 days.

Limousin x heifer  
300.5 kg  
26 month  
07/11/08



Meat Sample A

Simmental x Bull  
317.4 kg  
26 month  
07/11/08

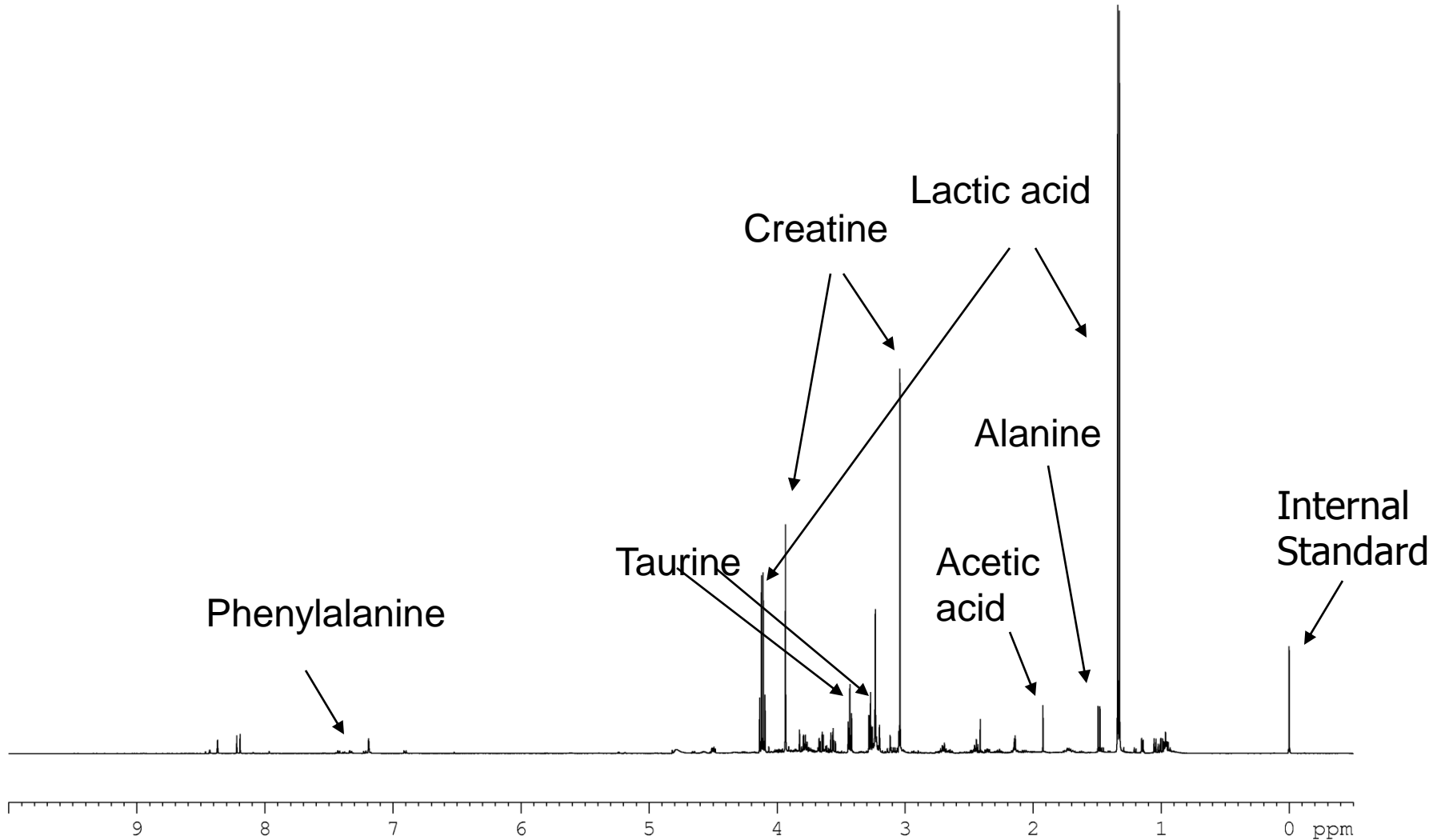


Meat Sample B

# Sample preparation

- 5 g of meat into 10 mL of water, homogenise, centrifuge.
- 1 mL of supernatant removed.
- 2 mL chilled acetone to remove protein.
- Centrifuge 14000 rpm 10 minutes.
- Supernatant lyophilised, resuspended in 800  $\mu\text{L}$  of  $\text{PO}_4$  buffer ( $\text{D}_2\text{O}$ ), with 1 mM  $\text{NaN}_3$  and 1 mM TSP. 600  $\mu\text{L}$  transferred to an NMR tube.

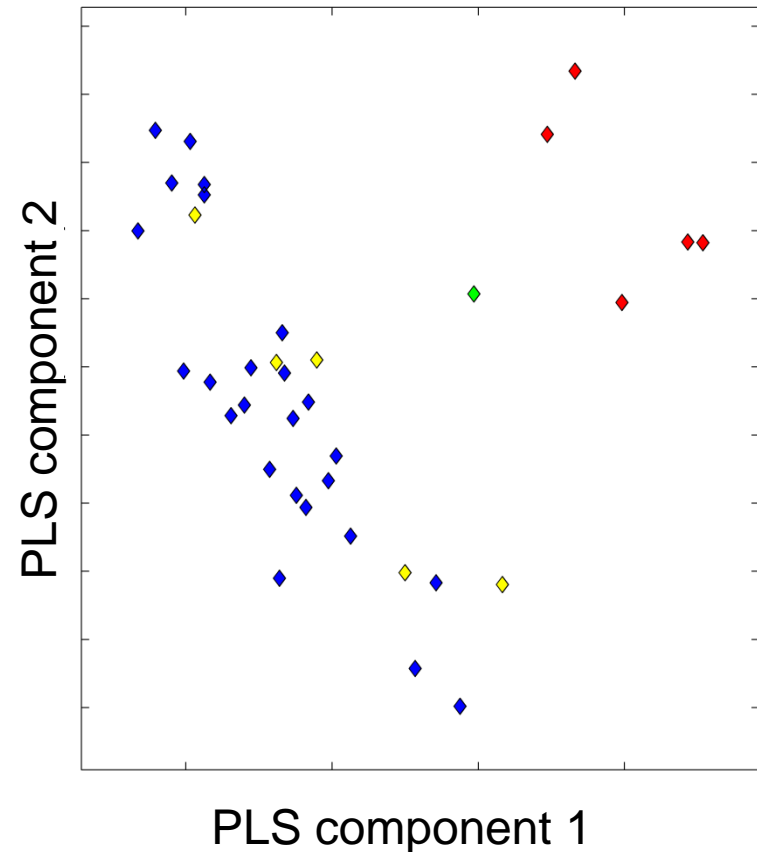
# $^1\text{H}$ NMR spectrum from meat extract



# PLS-LDA model construction

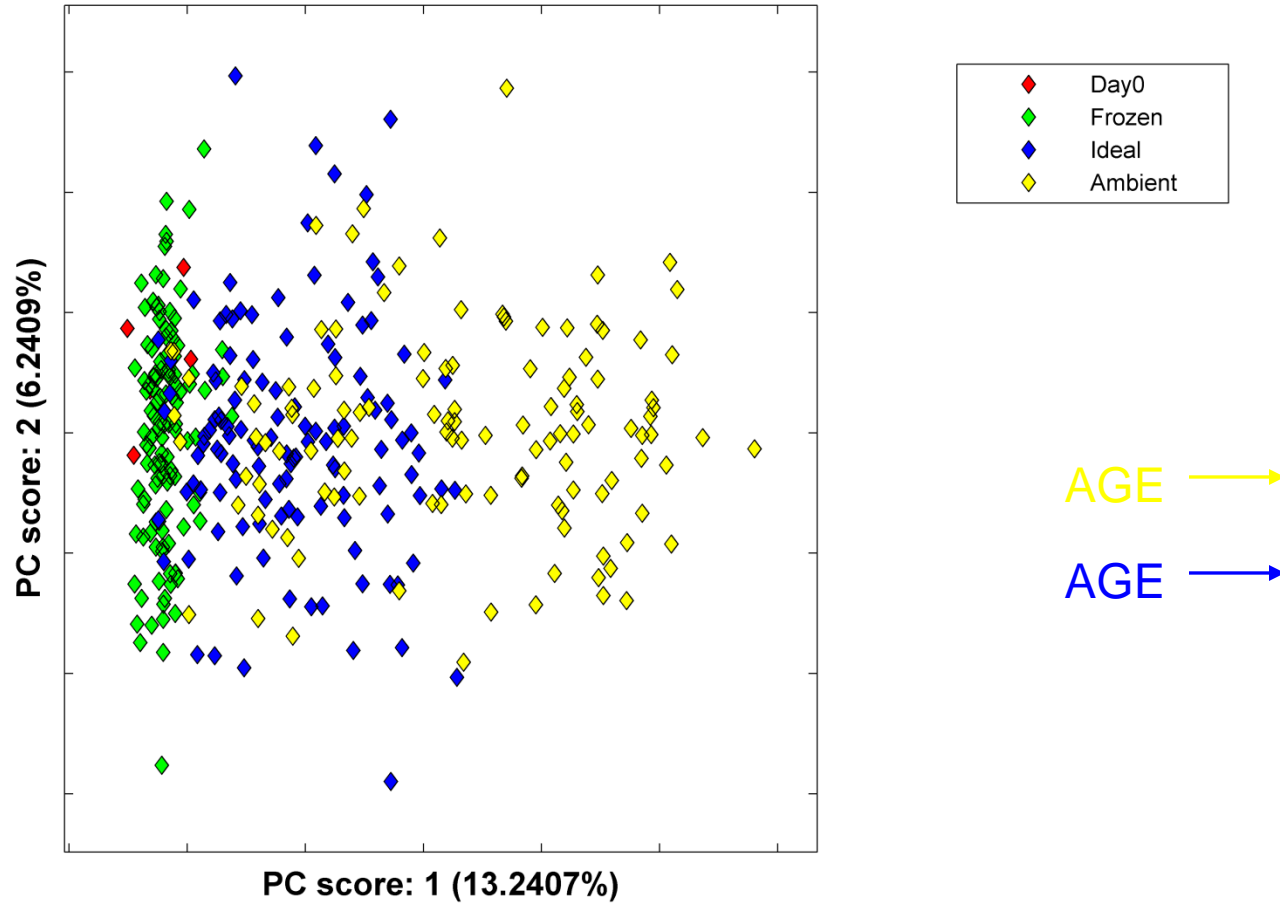
- PLS-LDA model built to determine if a meat sample had been aged 21 days.
- Sub-set of data chosen including samples which had not been aged, had been aged at the incorrect temperature and had been aged for longer than 21 days.
- Data split into a training ( $n = 30$ ) and a test set ( $n = 6$ ) to confirm validity of model.
- Model generated using training set and tested using the unseen test set.

# PLS-LDA model



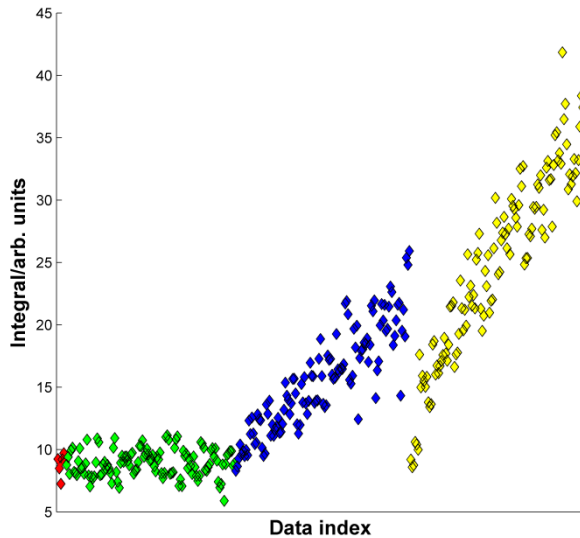
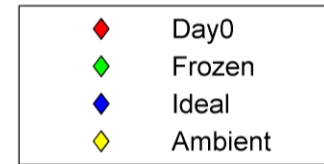
- ◆ Correctly stored and aged (training set)
  - ◆ Incorrectly stored and aged (training set)
  - ◆ Correctly stored and aged (test set)
  - ◆ Incorrectly stored and aged (test set)
- Model correctly classifies all samples correctly

# PCA (unit variance) – all data

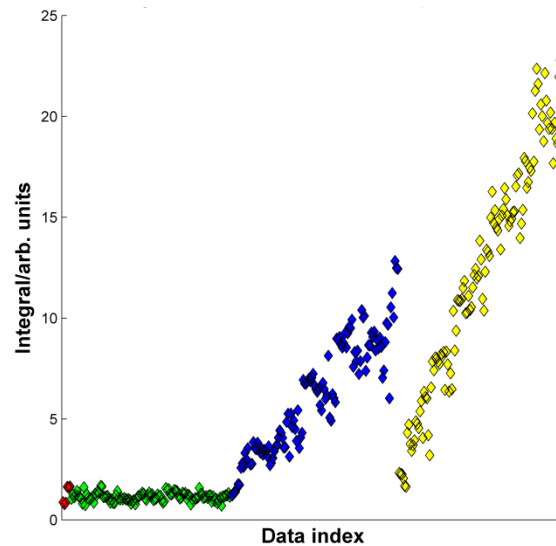


## Changes in $^1\text{H}$ NMR spectrum during storage

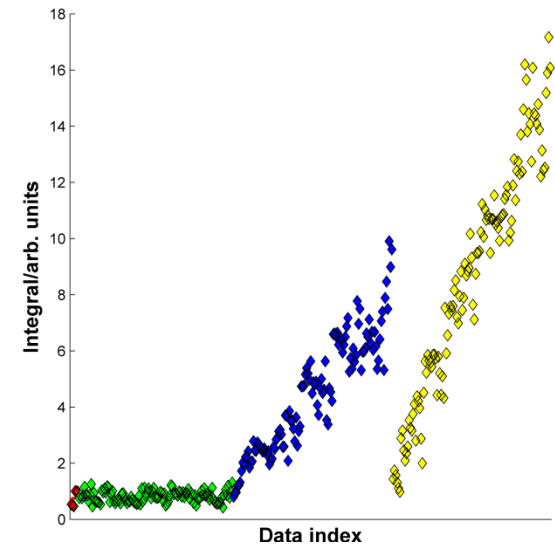
- Data sorted by storage condition and ordered by increasing age.



Alanine



Leucine

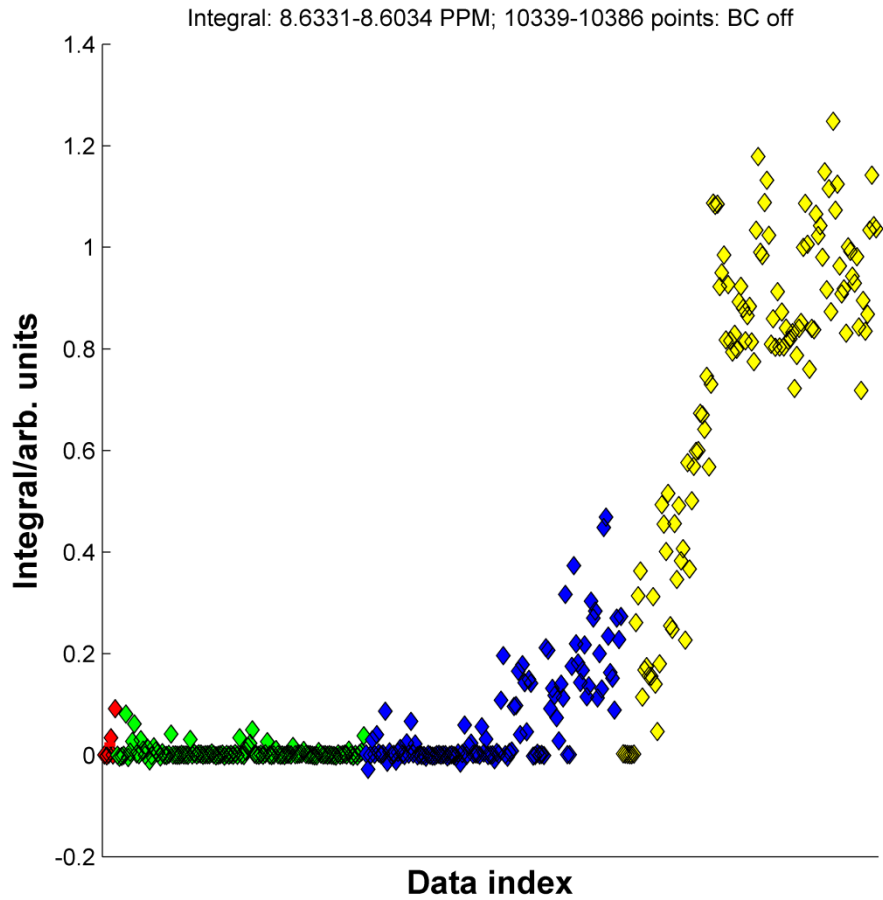
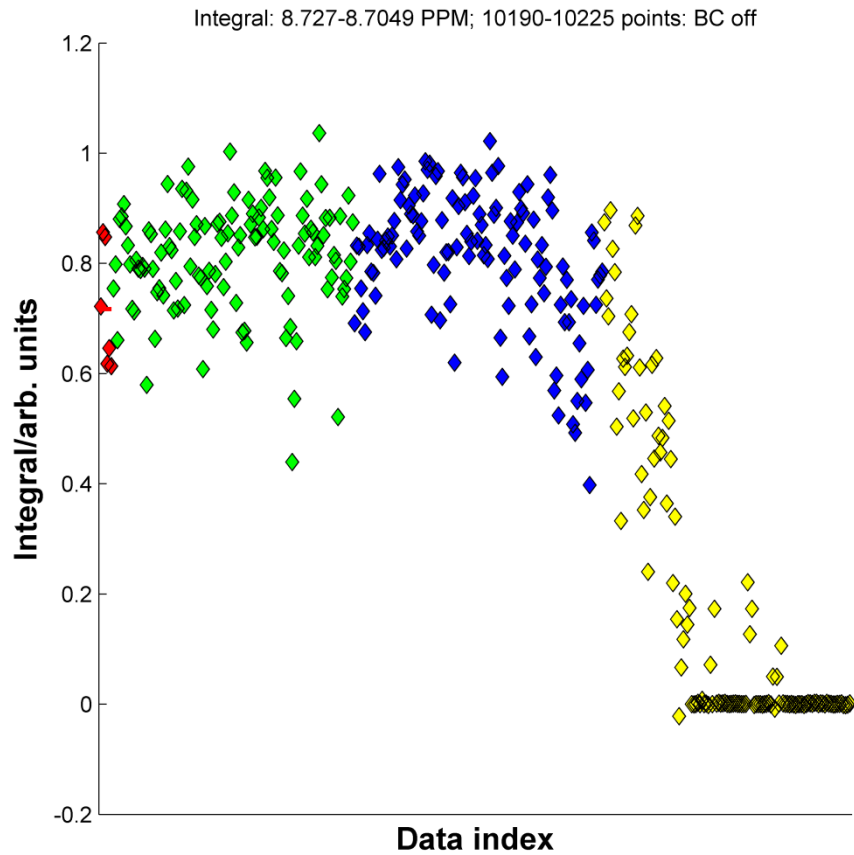


Phenylalanine

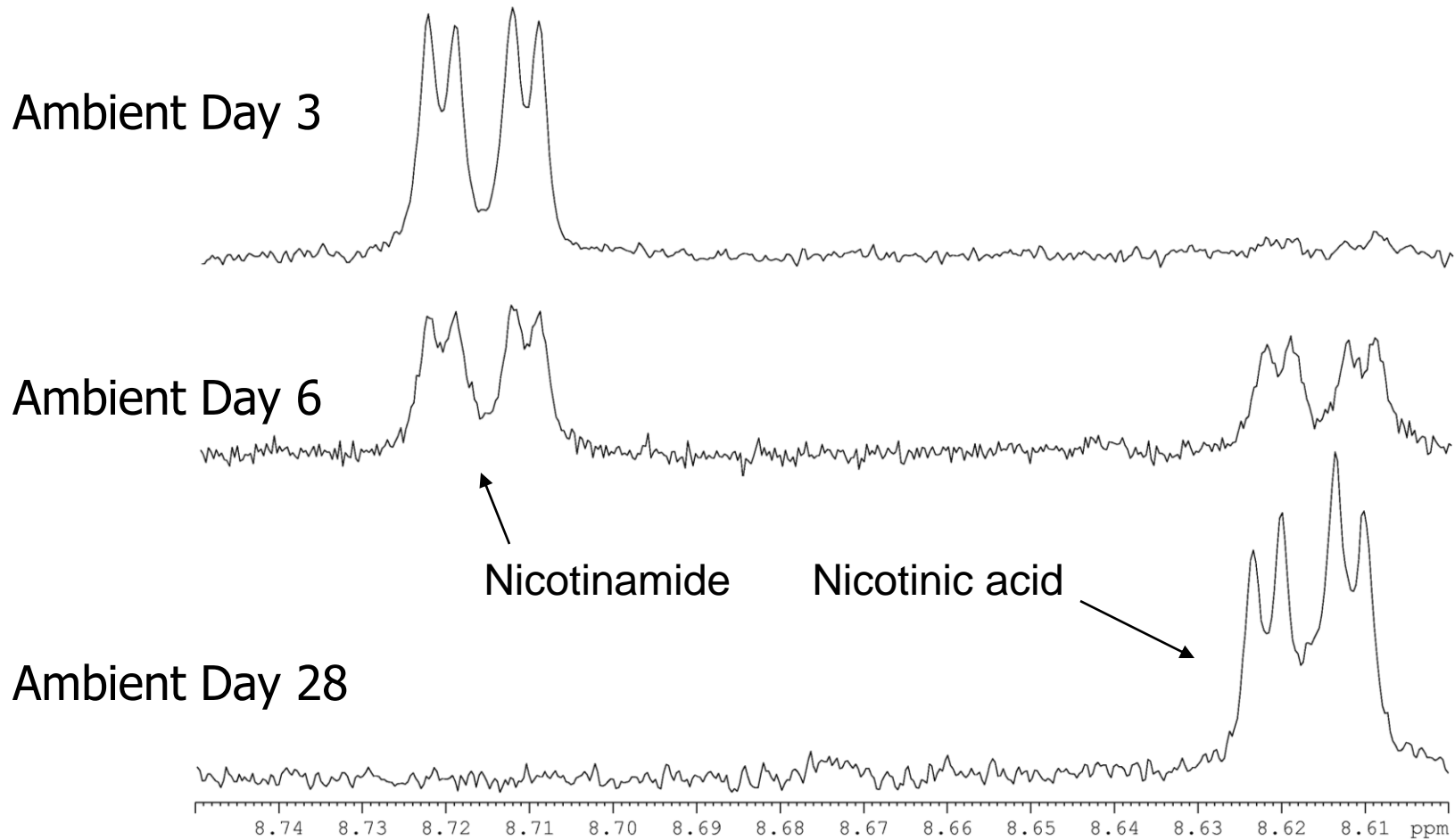
# Changes in $^1\text{H}$ NMR spectrum during

e

- ◆ Day0
- ◆ Frozen
- ◆ Ideal
- ◆ Ambient



# Changes in $^1\text{H}$ NMR spectrum during storage



## Conclusions

- $^1\text{H}$  NMR spectroscopic analysis is able to observe biological processes that occur during meat storage.
- A model built to predict corrected wet-aged samples was very successful.
- The concentration of specific amino acids increase linearly over time, and the rate of increase is related to storage temperature.
- Nicotinamide is converted to nicotinic acid at elevated temperatures. This process happens much slower in correctly stored samples.

# Acknowledgments

- Adrian Charlton, Steve Jones, Mark Harrison.
  - All members of TRACE Fingerprinting and Profiling Methods Workgroup (WP2).
  - EU and the Food Standards Agency.
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- Thank you for your attention  
Any Questions?