LowSalt project

WP1 food matrix and interactions

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WP1 description & aims

overall aim of the project

- to identify innovations that can reduce the NaCl level to 50 % of the current content of commercial products such as minced fish and meat, cured ham or smoked salmon.

NaCl reduction & substitution: focus on effects on:

- protein structural changes
- hydration properties of proteins – water interactions
- hydration properties of meat – WHC
- sensory properties / texture

methodology

- FTIR microscopy : existing platform + further development
- Raman microscopy
- NIR spectroscopy
- multivariate analysis

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WP1 milestones

M 1.1

- new methodology to study water and protein structures in a model system

M 1.2

- salt substitutes and/or water-binders which mimic protein-water matrix as obtained with high NaCl-concentrations (> 3 %) in a model system are identified

M 1.3

- salt substitutes and/or water-binders which mimic high salt-protein-water matrix in minced fish and meat systems are identified

M 1.4

- Testing of selected salt substitutes and/or water-binders in minced fish and meat products completed

M 1.5

- Testing of selected salt substitutes and/or low salt technologies in cured ham completed

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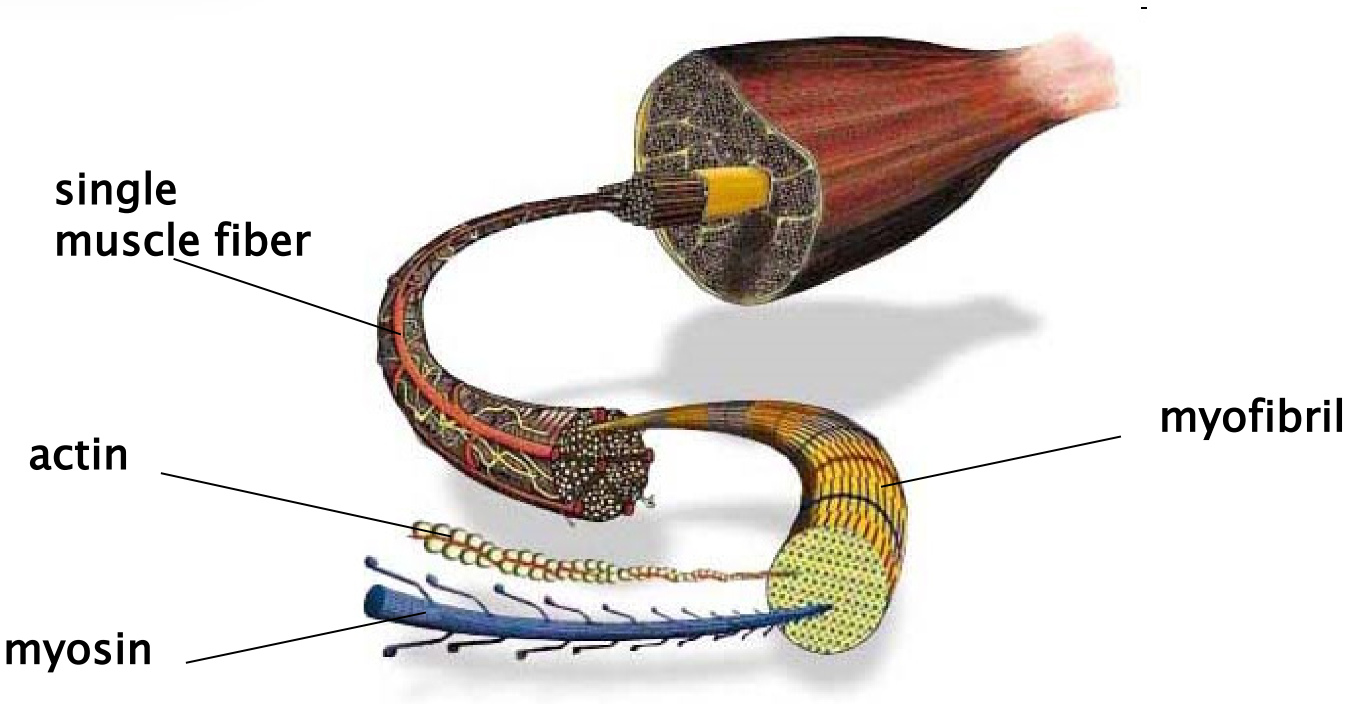
muscle organisation



meat

- water (~75%)
- **protein (~19%)**
- fat (~3%)
- soluble non-protein substances (~3.5%).

proteins

– actin – myosin	main constituents
– α-actinin – β-actinin – tropomyosin – troponin – C protein – M line protein	acessory proteins

background

protein structure & methodology

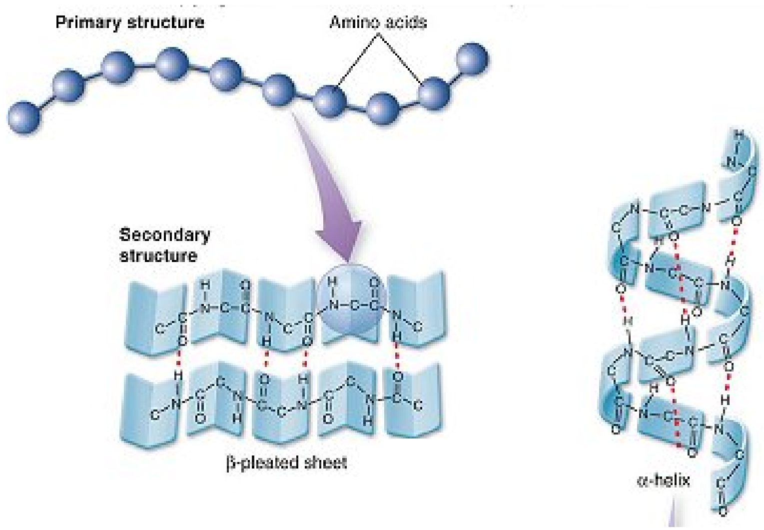
MOLECULAR STRUCTURE

Primary (sequence)

- order of the individual building blocks
- AA connected by peptide bond

background

protein structure & methodology



MOLECULAR STRUCTURE

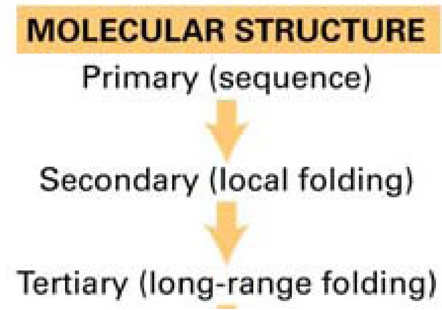
Primary (sequence)

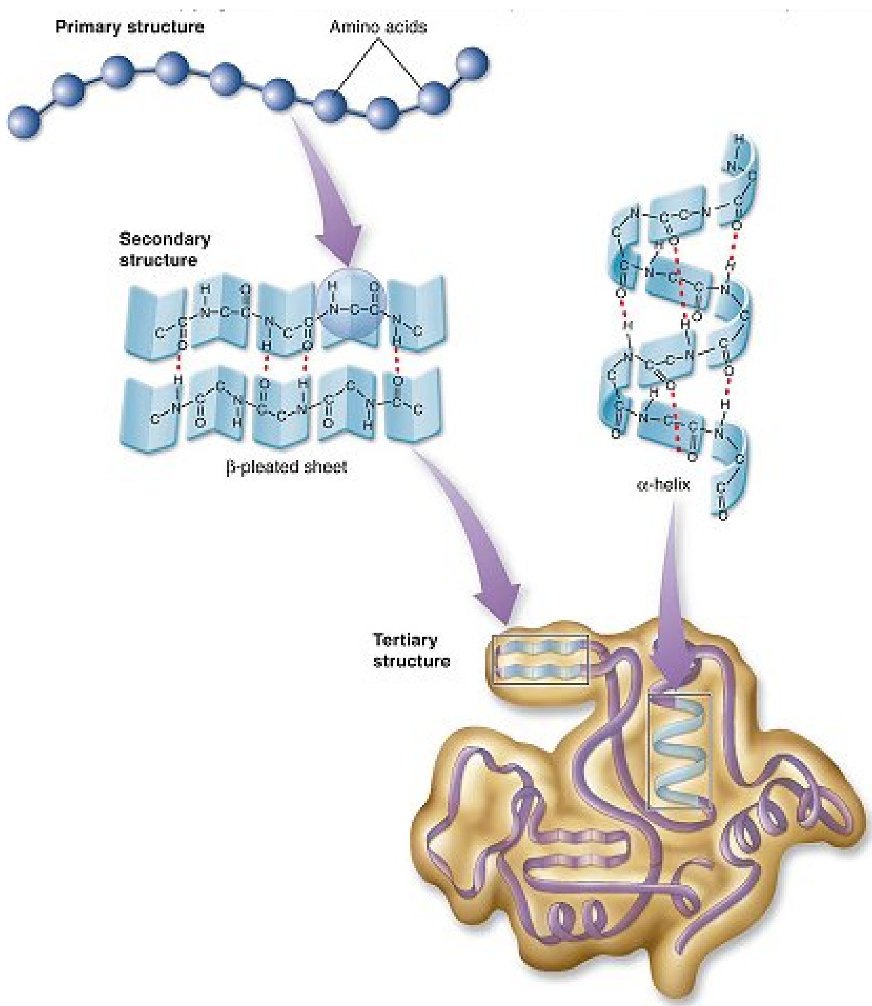
↓

Secondary (local folding)

- how these building blocks relate to each other
- stabilised by several interactions

protein structure & methodology





- how these fold in a 3D unit
- inter-relation between 2'' structures

protein structure & methodology

MOLECULAR STRUCTURE

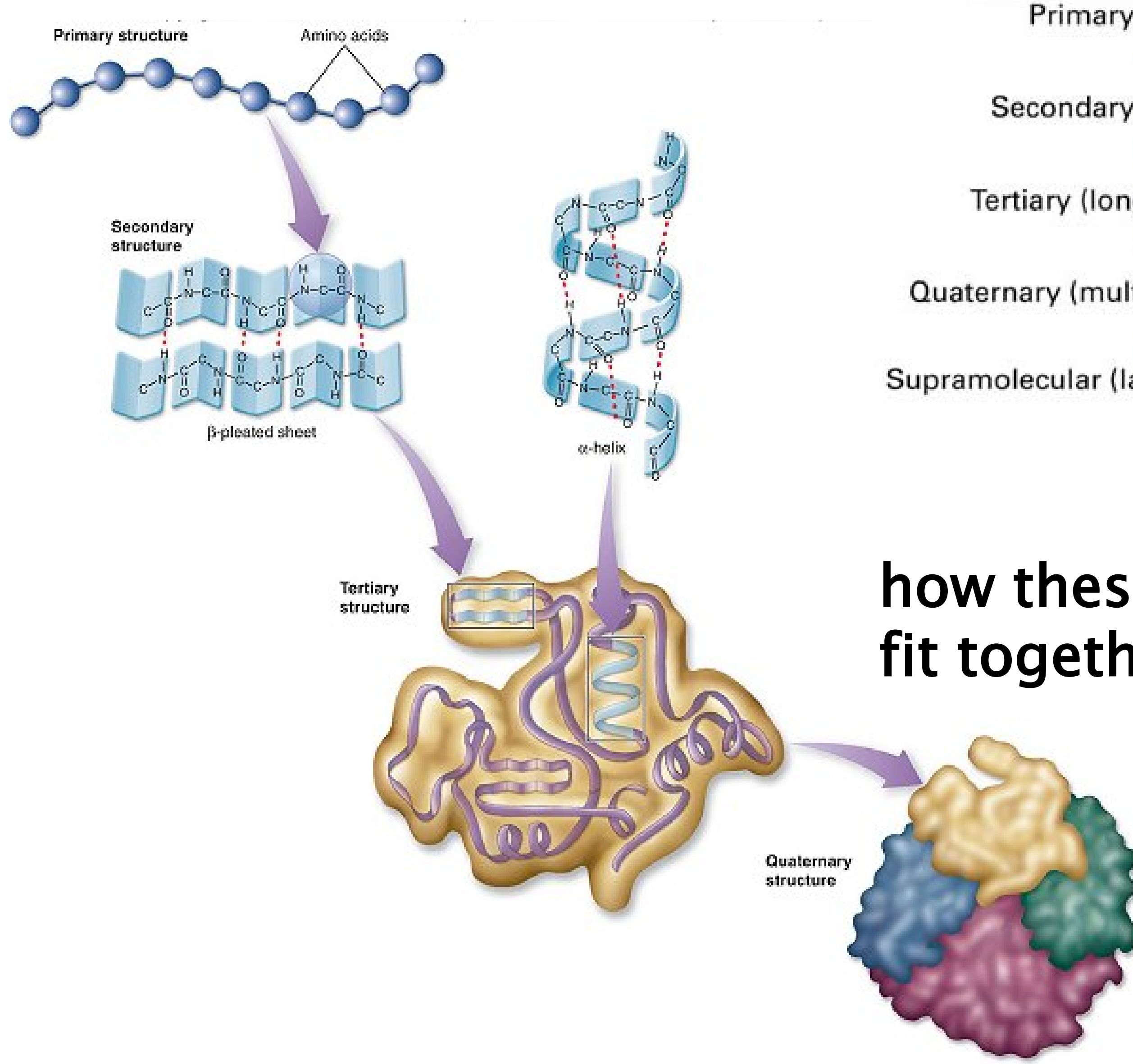
Primary (sequence)

Secondary (local folding)

Tertiary (long-range folding)

Quaternary (multimeric organization)

Supramolecular (large-scale assemblies)



how these units fit together

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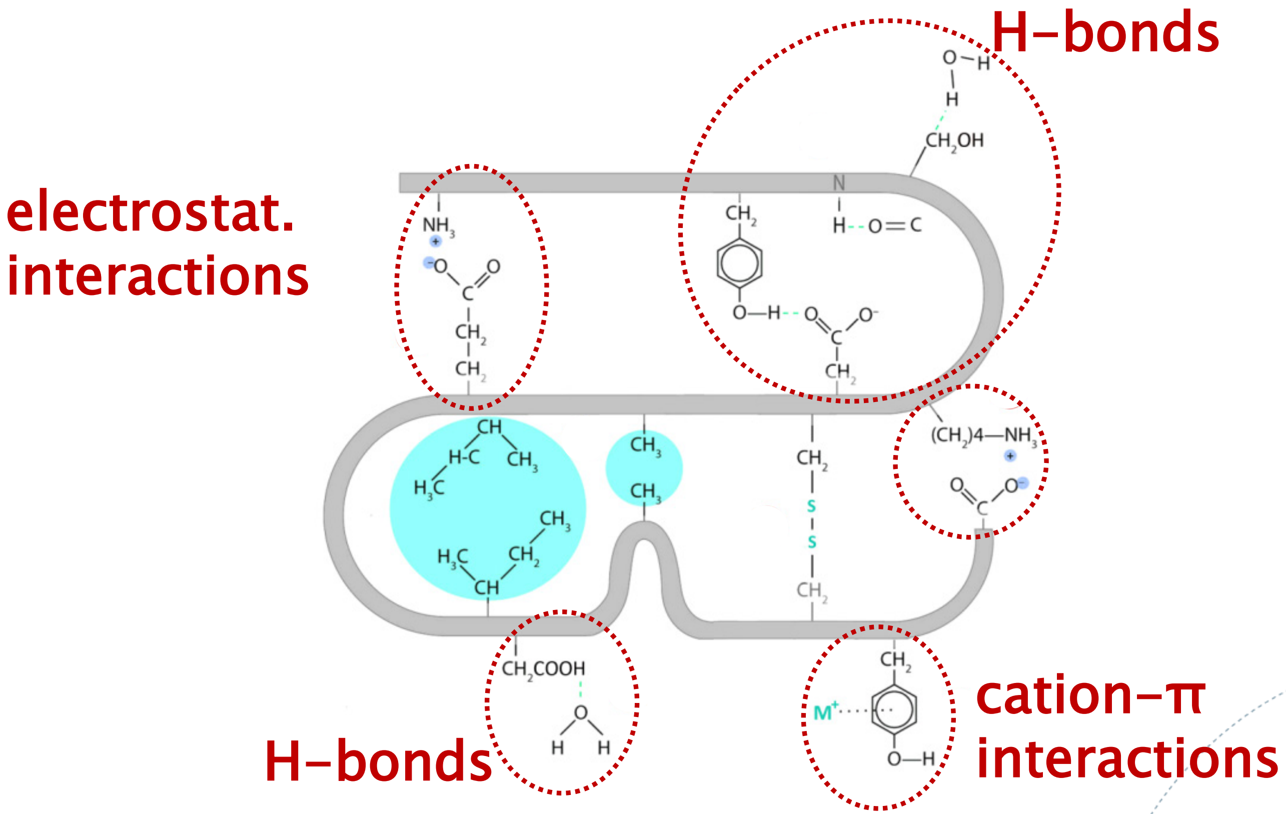
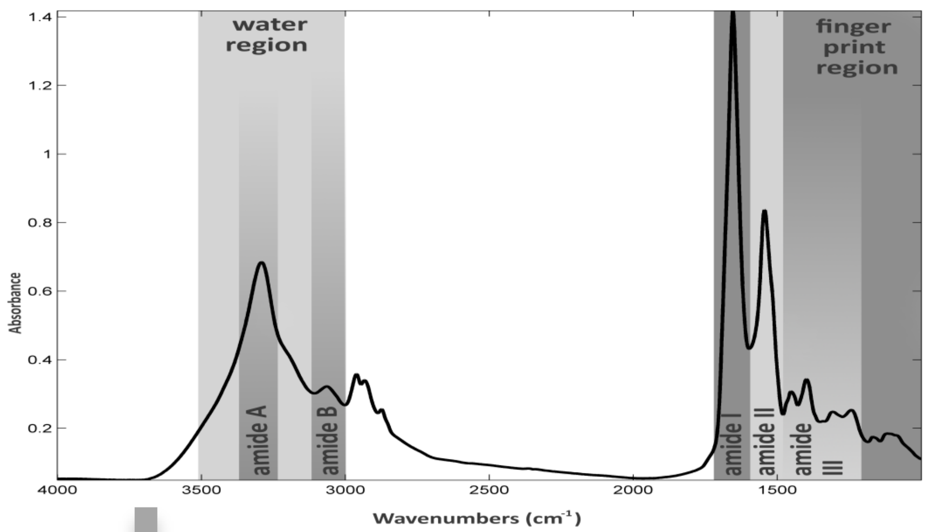
protein structure & methodology



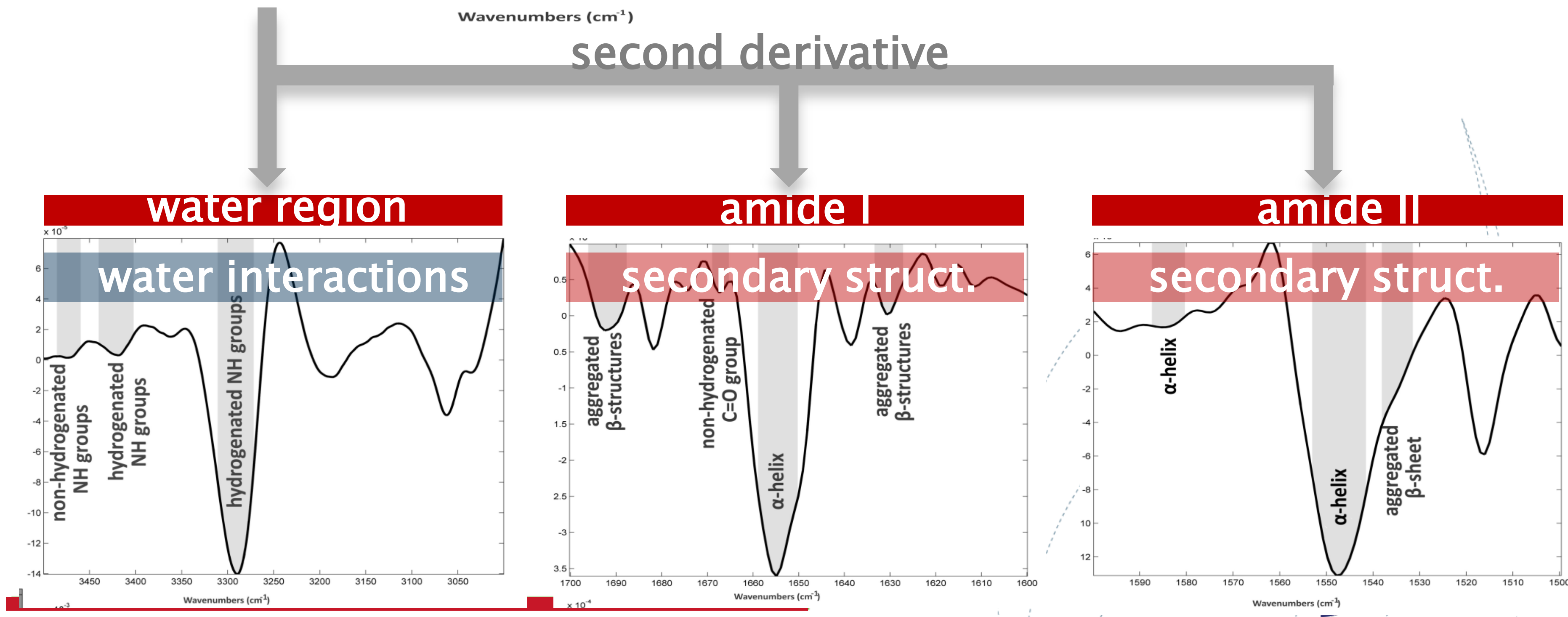
protein structure
stabilising forces
affected by salts

protein structure
stabilising forces

protein structure & methodology



myofiber FTIR spectrum



outline – publications

Experiment 1 → paper 1, paper 2

- method improvement
- model system
- structure analysis

Experiment 2 → paper 3

- improved methods
- real system –**meat**
- structure analysis

Experiment 3 → paper 4

- improved methods
- real system –**sausage**
- structure analysis

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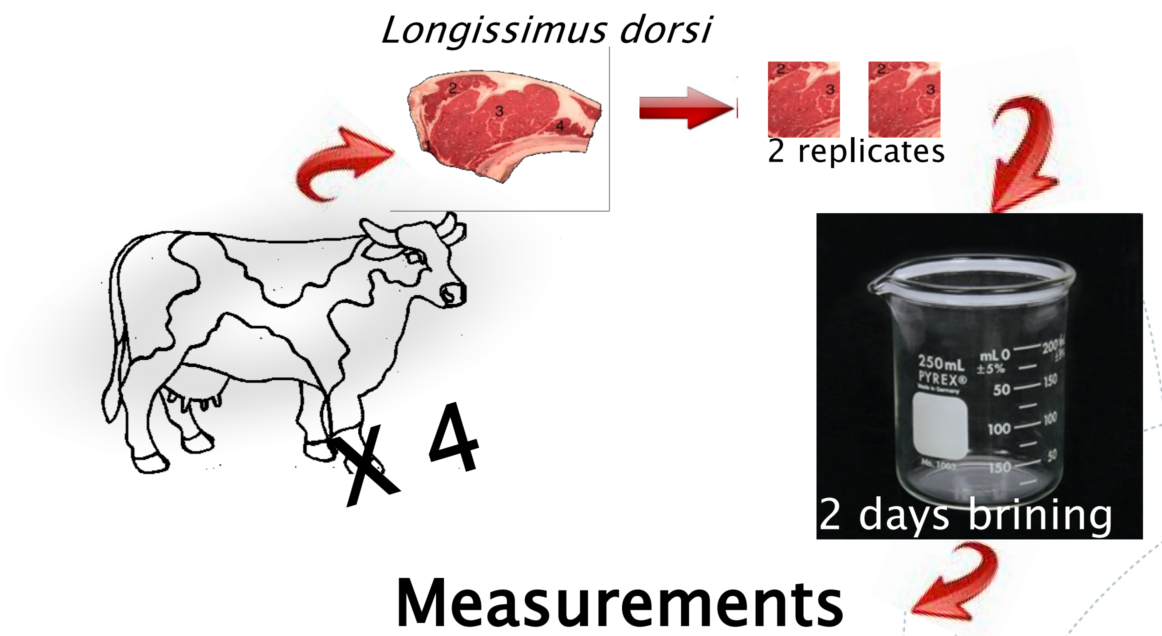
Experiment 1

- 3 salts – **NaCl** / **KCl** / **$MgSO_4$**
- 3 concentrations – **1,5** % / **6** % / **9** %
- 4 animals (beef)
- 3 spectroscopic methods– **FTIR, Raman, NIR**



Paper 1

Monitoring Protein Structural Changes and Hydration in Bovine Meat Tissue Due to Salt Substitutes by Fourier Transform Infrared (FTIR) Microspectroscopy

Journal of Agricultural and Food Chemistry

Perisic, N., Afseth, N. K., Ofstad, R., & Kohler, A. Monitoring Protein Structural Changes and Hydration in Bovine Meat Tissue Due to Salt Substitutes by Fourier Transform Infrared (FTIR) Microspectroscopy. *Journal of Agricultural and Food Chemistry*, 2011, *59*(18), 10052–10061.

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Step 1: literature overview– band assignement

Table 1. Band Positions and Assignments for the Amide I, Amide II, and Water Regions According to the Literature and Our Previous Work

region	freq (cm^{-1})	tentative assignment[a]
amide I	1693	aggregated β-sheet structures (sideband of 1631 cm^{-1} band),[32−35] M/P
1700−1600 cm^{-1}	1682	native (parallel/antiparallel) β-sheet structures,[20,32,33,37] M/P/T
(80% C=O stretch,	1674	tentatively assigned to turns,[22] M/P
10% C—N stretch,	1667	nonhydrogenated C=O group, internal random coil segments that are not involved in H-bonding,[33,35] M/P
10% N—H bend)	1660	loop structures/α-helical structures,[16,43,51] M/P
	1655	α-helical structures, C=O stretching vibrations originating from α-helical structures in the myofibrillar proteins,[20,32,33,37] M/P/T or water vibration,[36,38] P
	1638	water deformation mode in liquid water [19,52] P or native (parallel/antiparallel) β-sheet structures,[22,33] M/P/T
	1631	aggregated β-sheet structures,[32−35] M/P
	1618	aggregated β-sheet structures,[16,43,51] M
	1611	tyrosine amino acid side chain vibrations[33,40] or aggregated strands,[41] M/P
amide II	1594	not assigned
1600−1500 cm^{-1}	1584	α-helical structures,[33] M
(60% N—H bend,	1575	amide II unspecified,[33] M
40% C—N stretch)	1567	residue and/or possibly aggregated β-sheet structures,[33,42] M
	1547	α-helical structures,[33] M/P
	1537	possibly aggregated β-sheet structures,[33] M
	1516	possibly tyrosine,[33] M/P
water region	3473	nonhydrogenated N—H groups,[19,22,39] P
3500−3000 cm^{-1}	3420	hydrogenated N—H groups or O—H stretching band,[21,22,39,53] T/P
(N—H stretching,	3361	companion band of 1530 cm^{-1} band, in solution occurring at 3345 cm^{-1}, and/or N—H stretching band,[21,39] T/P
C—N—H stretching vibration,	3290	N—H stretching band/amide A,[21,22,39,42] T/P, or hydrogen-bonded NH groups [19] P
O—H stretching vibration)	3190	not assigned
	3063	N—H stretch/amide B/amide II overtone/amide II combination mode in β-sheet structures,[22,39] T/P
	3035	not assigned

[a] M, obtained in real meat system; P, obtained in pure protein or polypeptide model system; T, obtained by theoretical calculation and/or prediction.

secondary structure

water interact

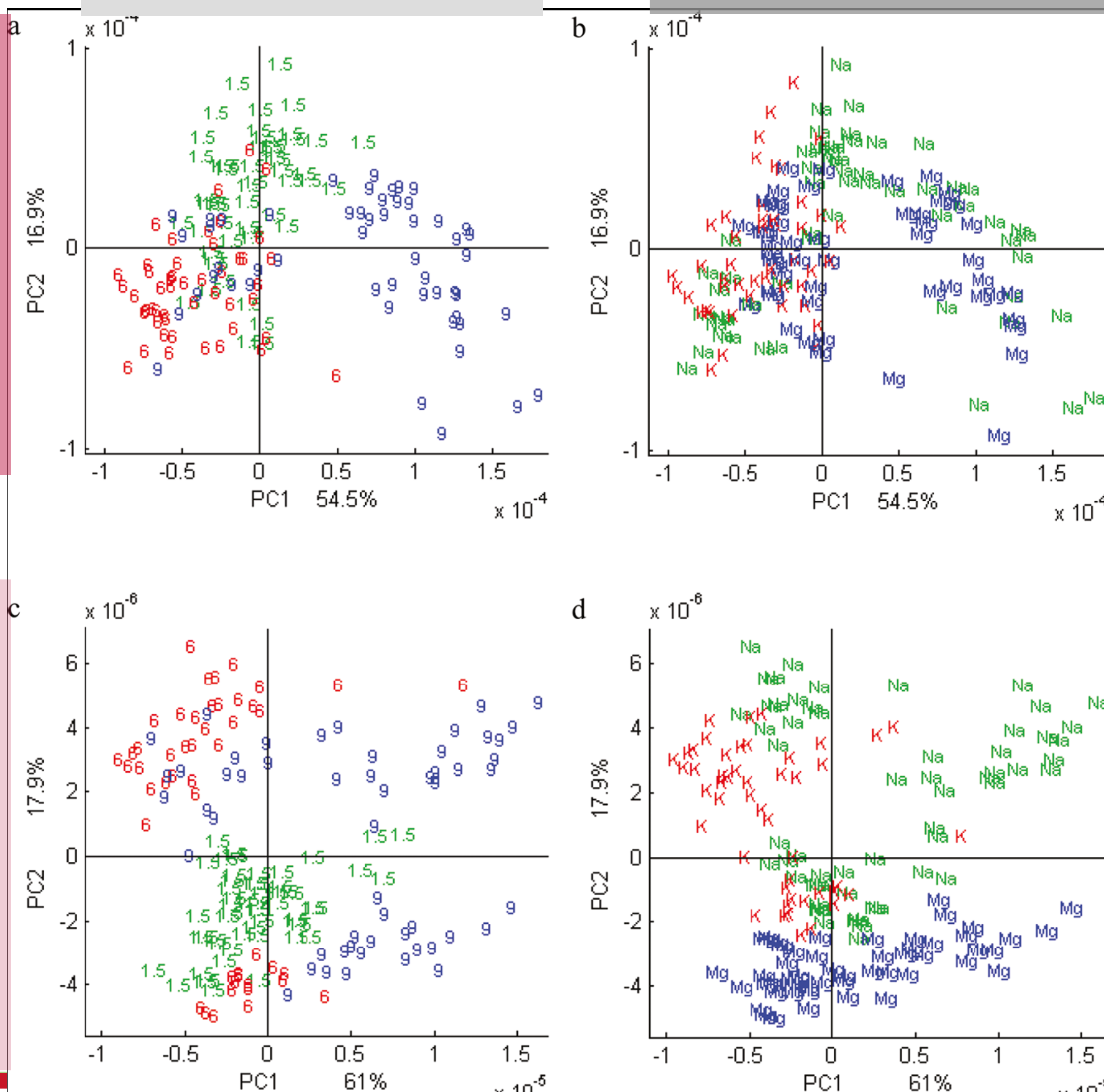
Step 2: PCA of separate regions

concentration

salt type

protein secondary structure

protein water interactions



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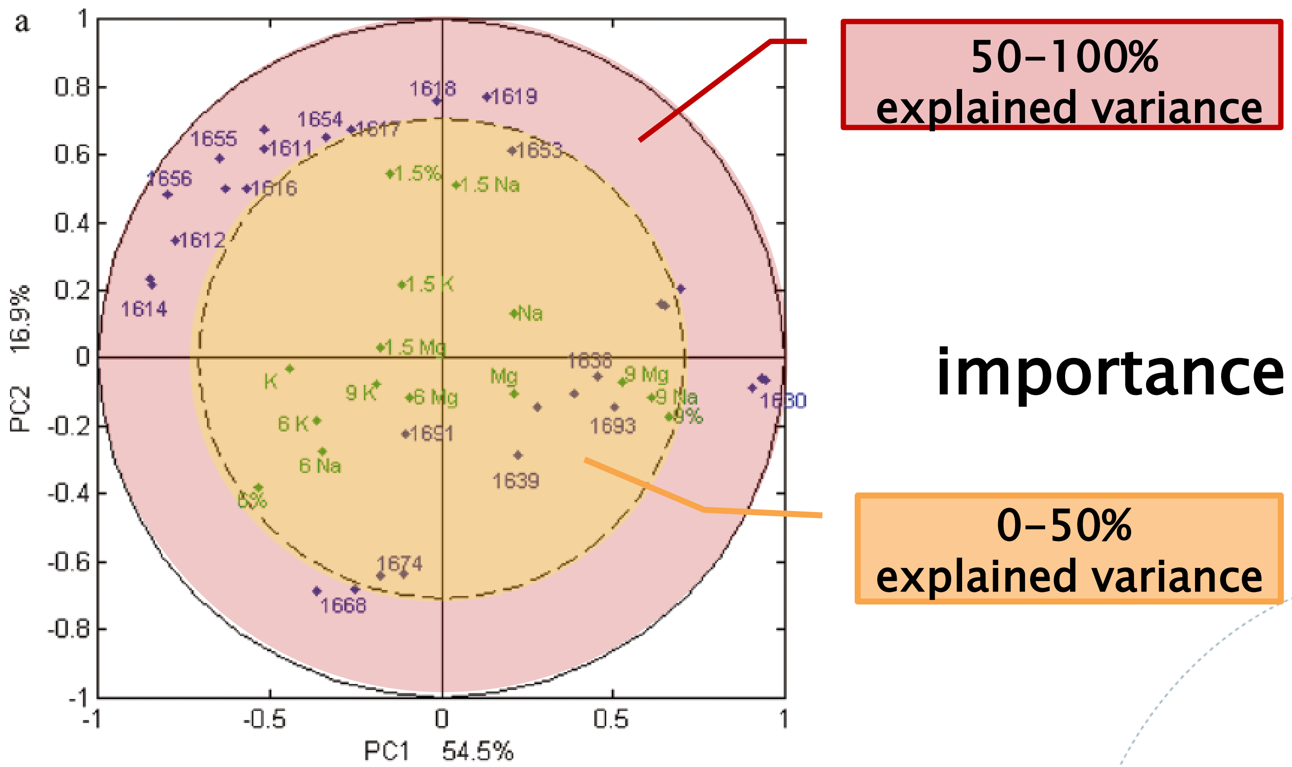
Step 2: PCA of separate regions
correlation loading plots: relating to design variables



importance of a variable

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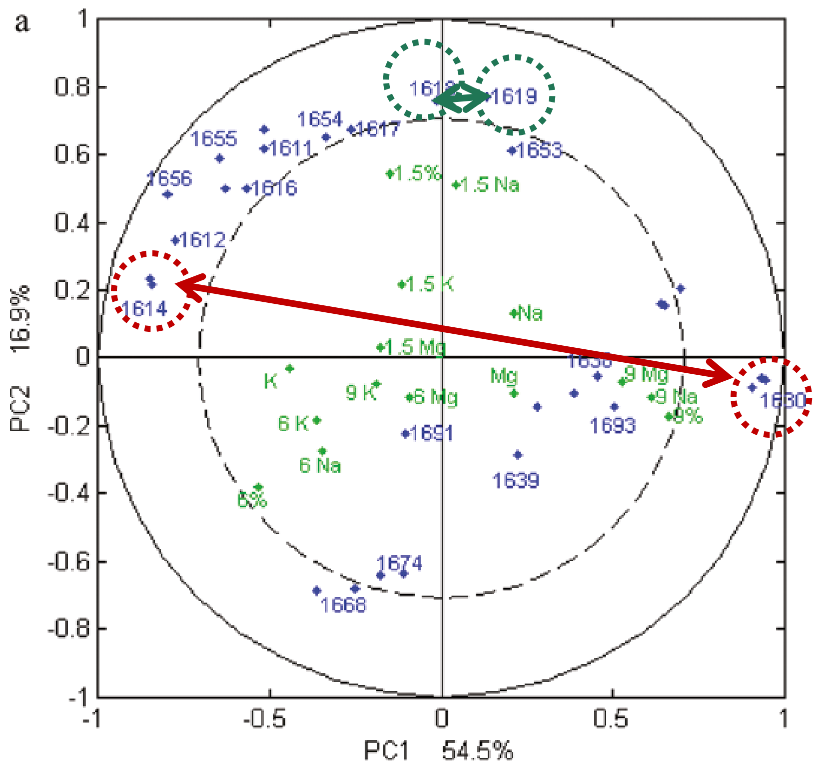
Step 2: PCA of separate regions
correlation loading plots: relating to design variables

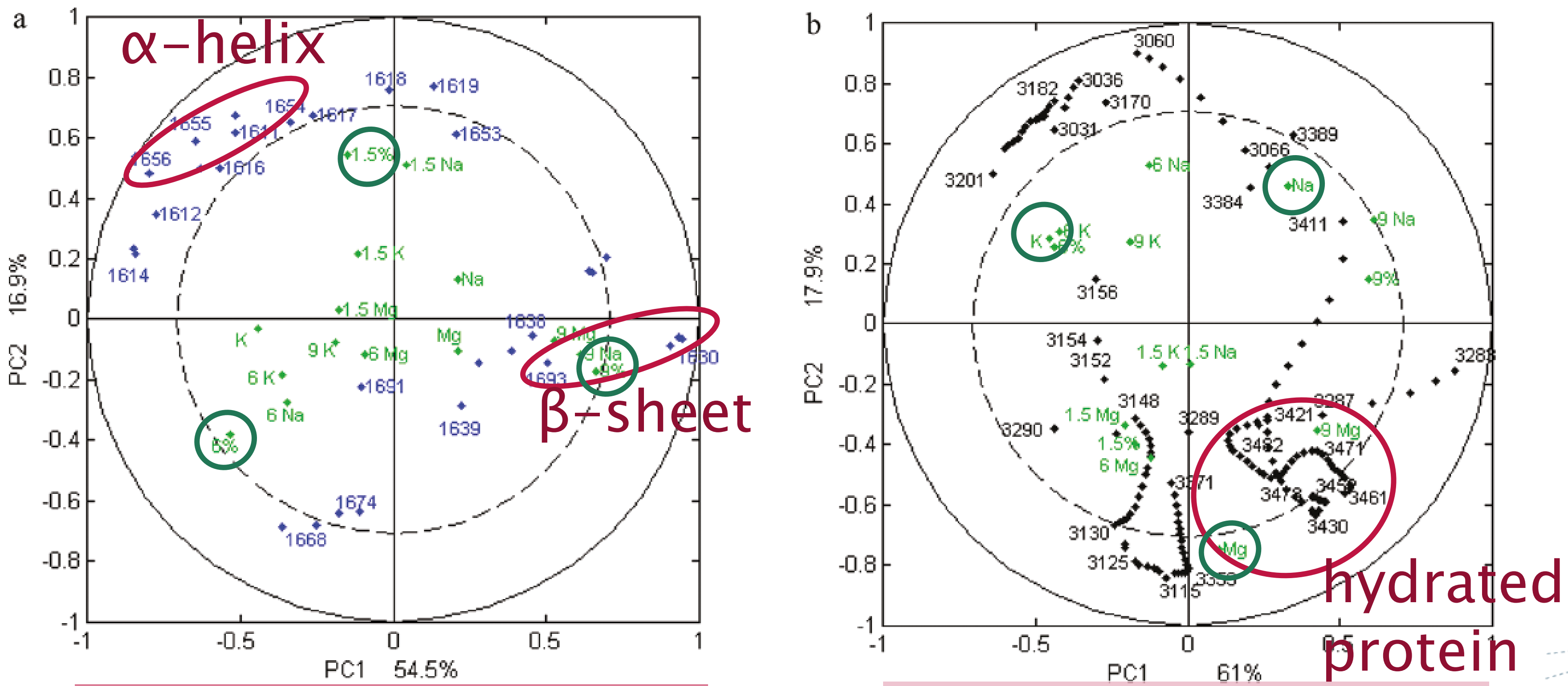


positive correlation

correlation between variables

negative correlation

Step 2: **PCA** of separate regions
correlation loading plots: relating to design variables



protein secondary structure

protein water interactions

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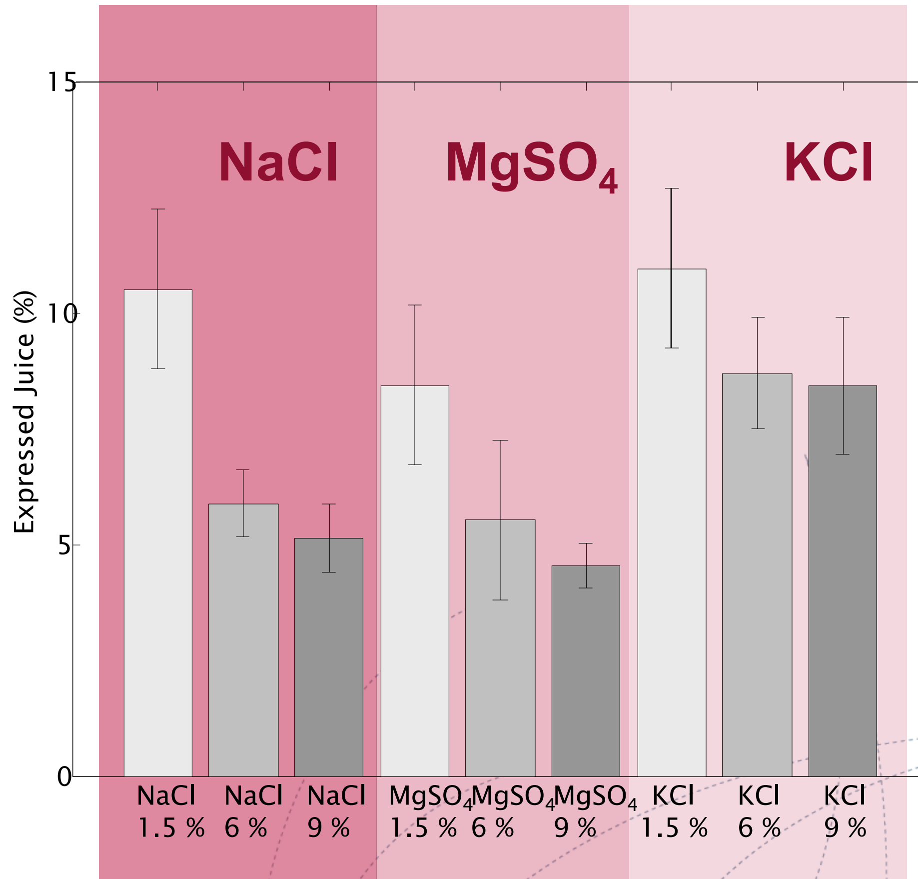
Step 4: water holding capacity

WHC ↑=↓ juice

- additional measurements (commercial meat samples)
- expressed juice method

- supportig **FTIR** results:
  - – **$MgSO_4$** – highest WBC
  - – simillar to NaCl

  - – **KCl** – lowest WBC
  - – different than NaCl



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Paper 2

Characterising protein, salt and water interactions with combined vibrational spectroscopic techniques

Food Chemistry

Perisic, N., Afseth, N. K., Ofstad, R., Hassani, S. & Kohler, A. Characterising protein, salt and water interactions with combined vibrational spectroscopic techniques. *Food Chemistry*, 2012, *in press*

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Paper 2

Characterising protein, salt and water interactions with combined vibrational spectroscopic techniques

- FTIR microscopy
- Raman microscopy
- NIR spectroscopy

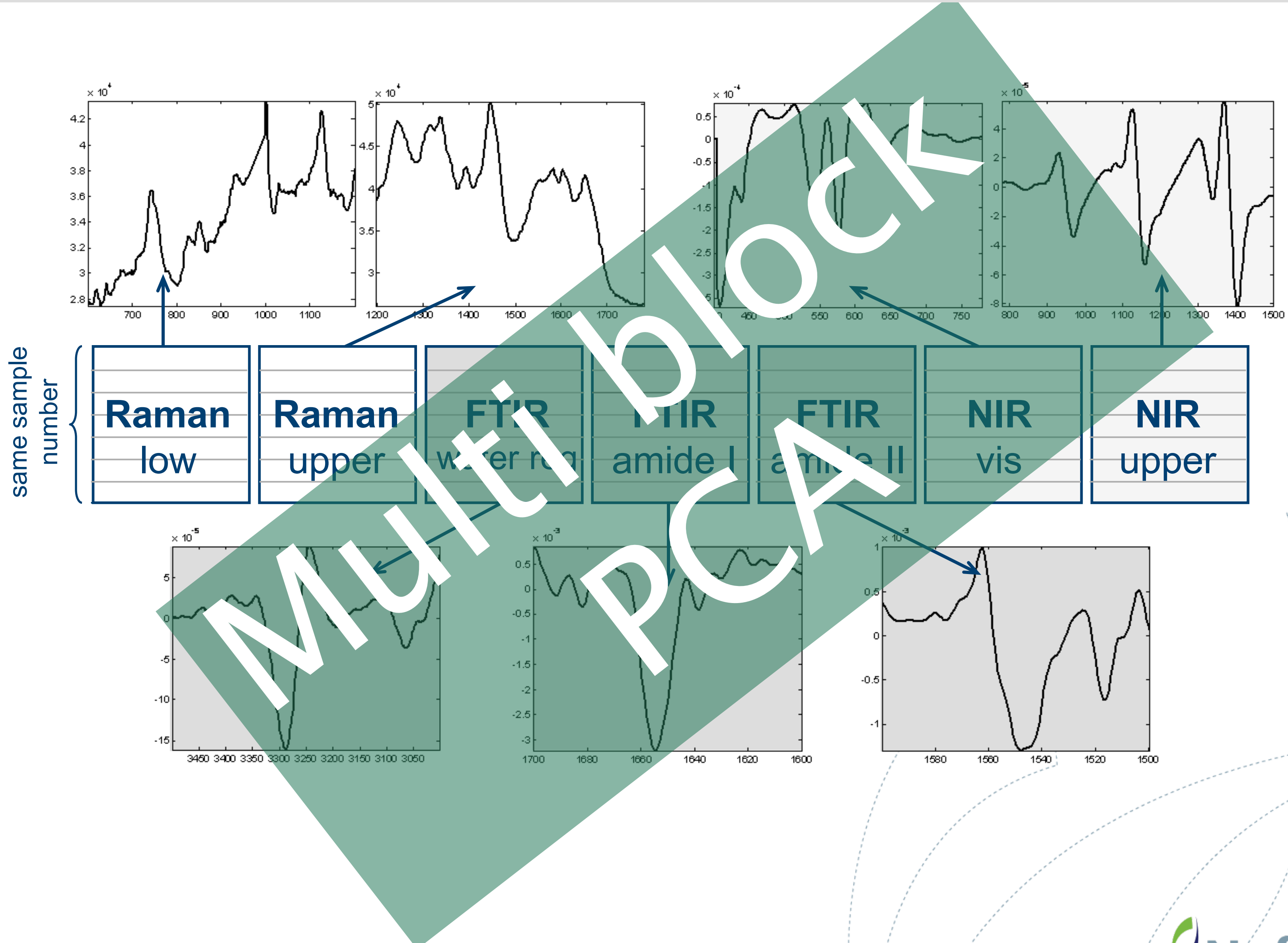
find information

characteristic for each method

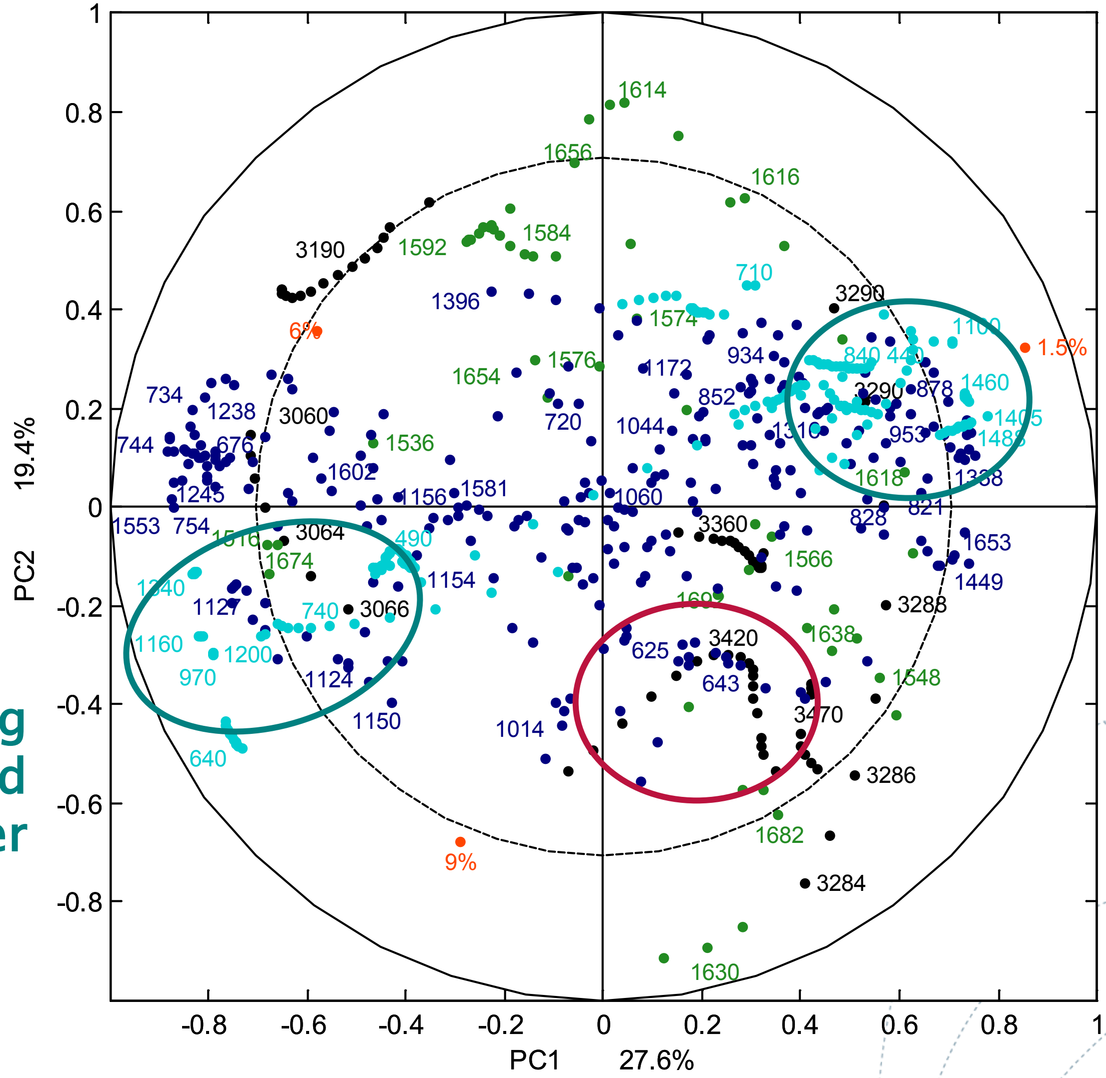
&

common between methods

resulting data set



MB-PCA: correlation loading plot



loose water

H-bonding bound water

AA residue (Raman)

& hydrogenated Prot (FTIR)

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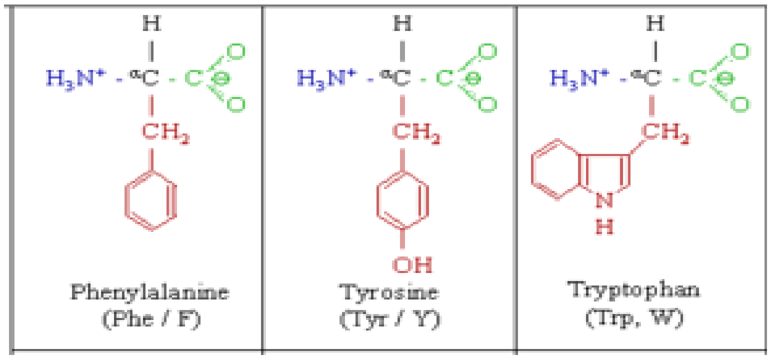
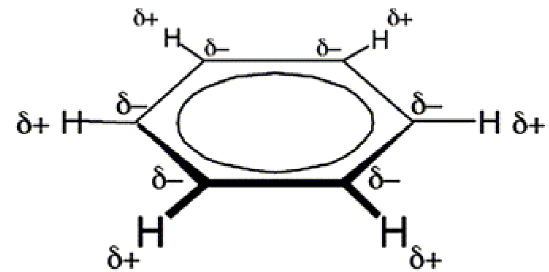
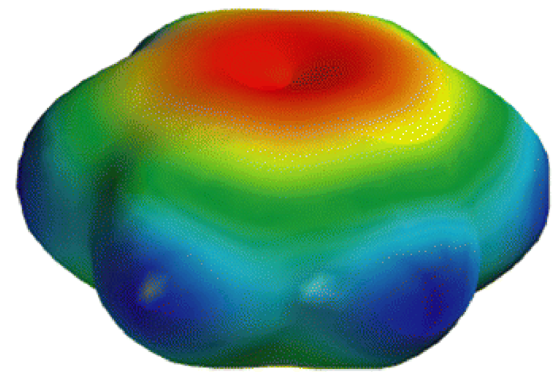
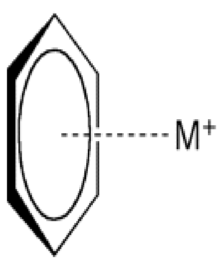
Cation–π interactions: involving aromatic amino acids

AA residues with aromatic ring

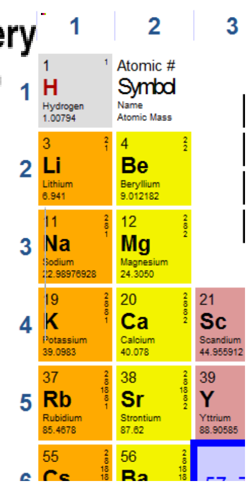








M+	Binding Enery (kcal/mol)
Li^+	38
Na^+	28
K^+	19
Rb^+	16

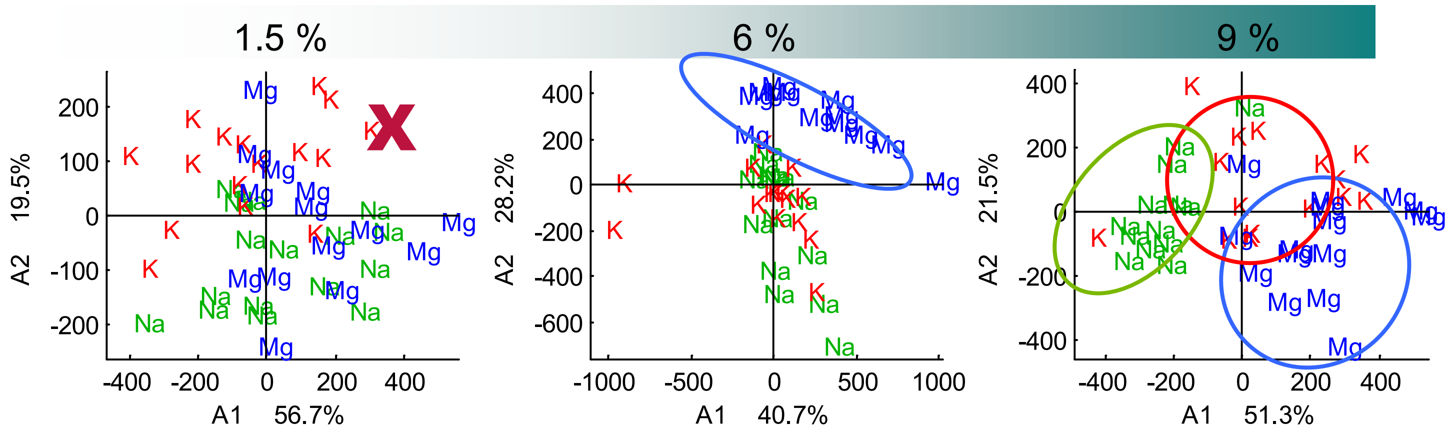


Gas phase binding energies (–ΔH) for alkali metals to benzene.

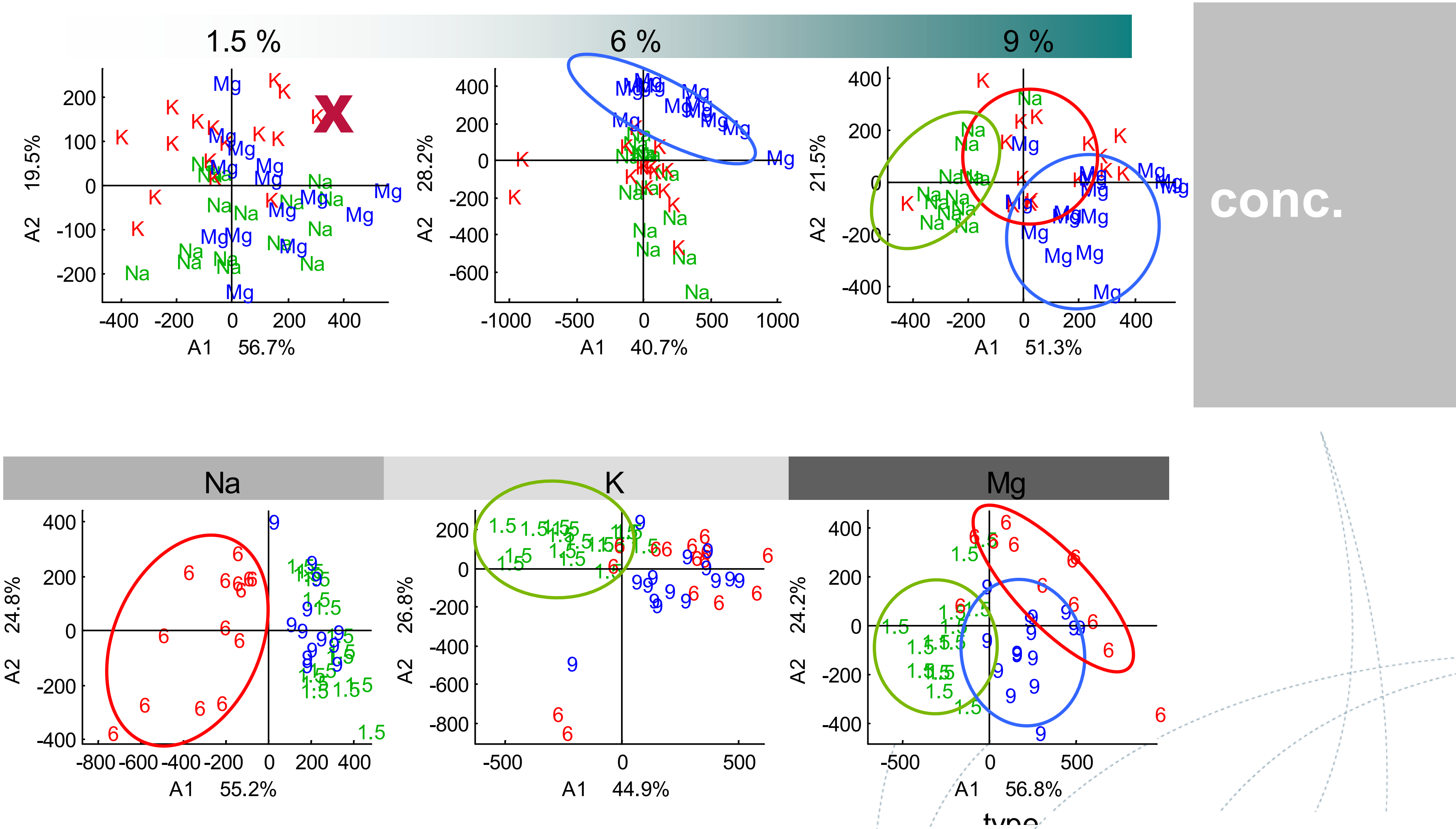
- ◆ positive charge
- ◆ negative charge

J. Nutr. June 1, 2007 vol. 137 no. 6 1504S-1508S

PCA: Raman AA residue bands



PCA: Raman AA residue bands



Paper 3

Characterizing salt substitution in beef meat processing by vibrational spectroscopy and sensory analysis

Meat Science

Perisic, N.; Afseth, N. K.; Ofstad, R.; Narum, B.; Kohler, A.; Characterizing salt substitution in beef meat processing by vibrational spectroscopy and sensory analysis

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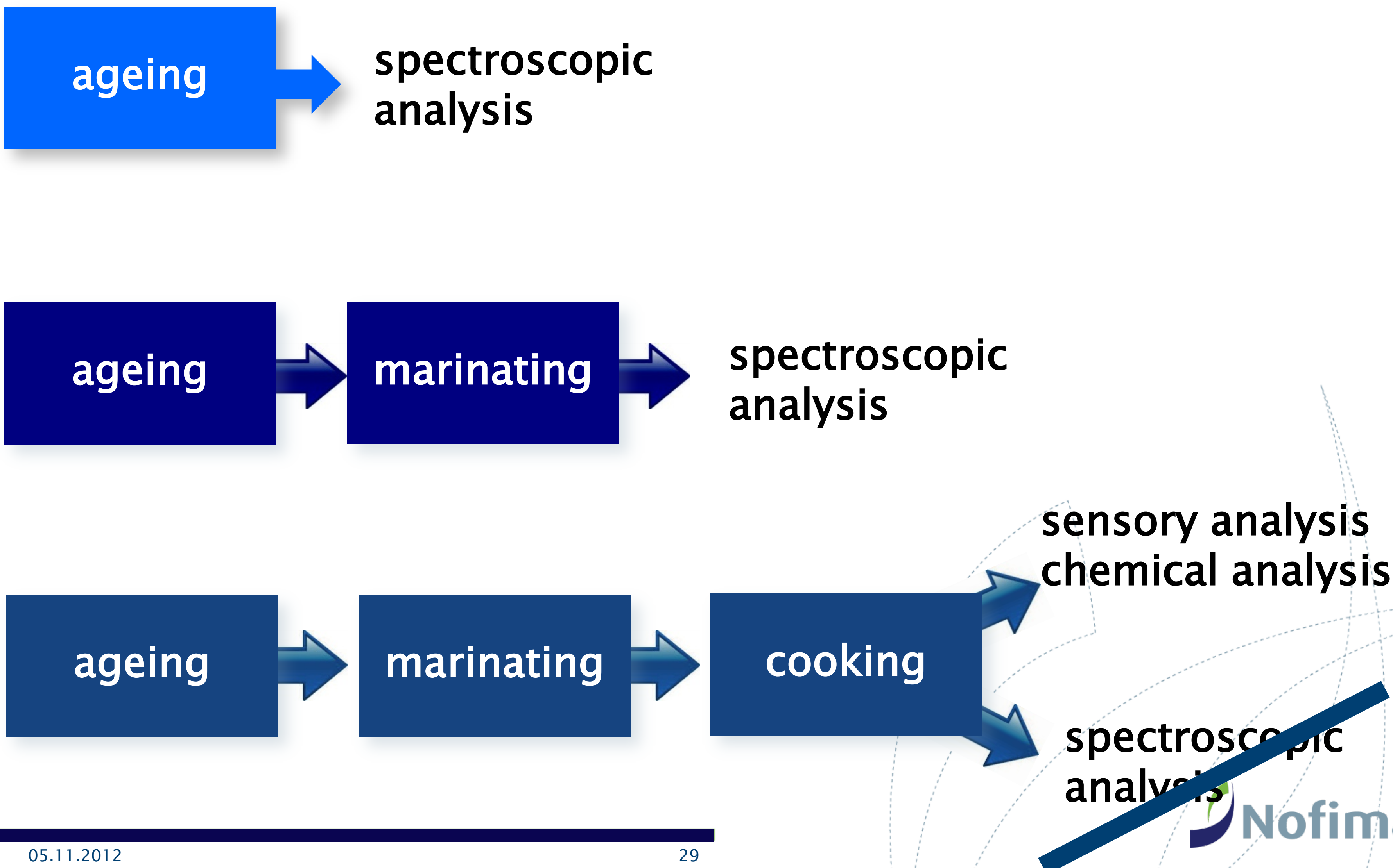
Characterizing salt substitution in beef meat processing by vibrational spectroscopy and sensory analysis

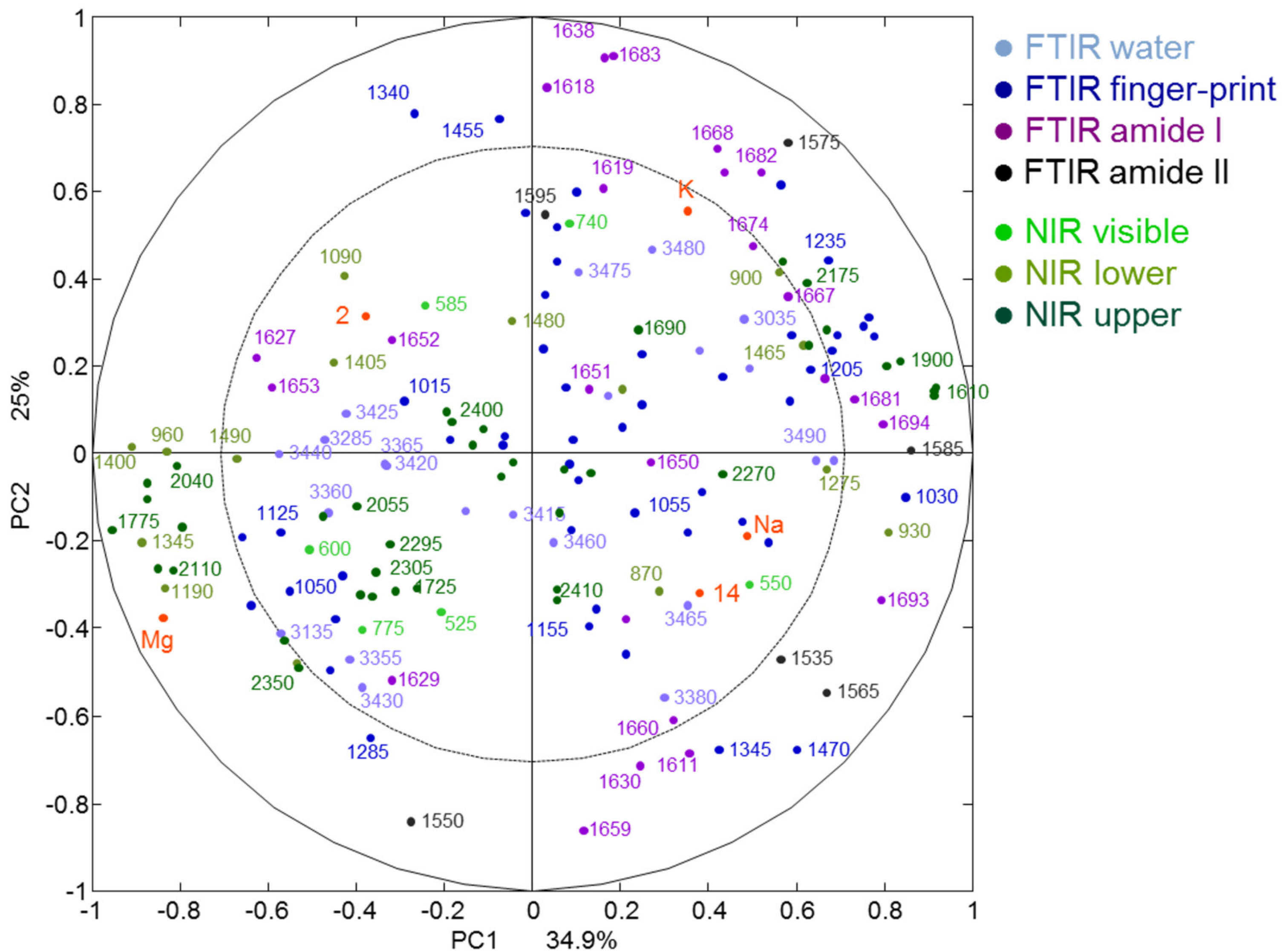
Experiment 2

- 3 salts – **NaCl** / **KCl** / **$MgSO_4$**
- 1 concentrations – **5,5** % (in marinade)
- 4 animals (beef)
- heat treatment
- ageing
- 2 spectroscopic methods– **FTIR, NIR**
- sensory analysis

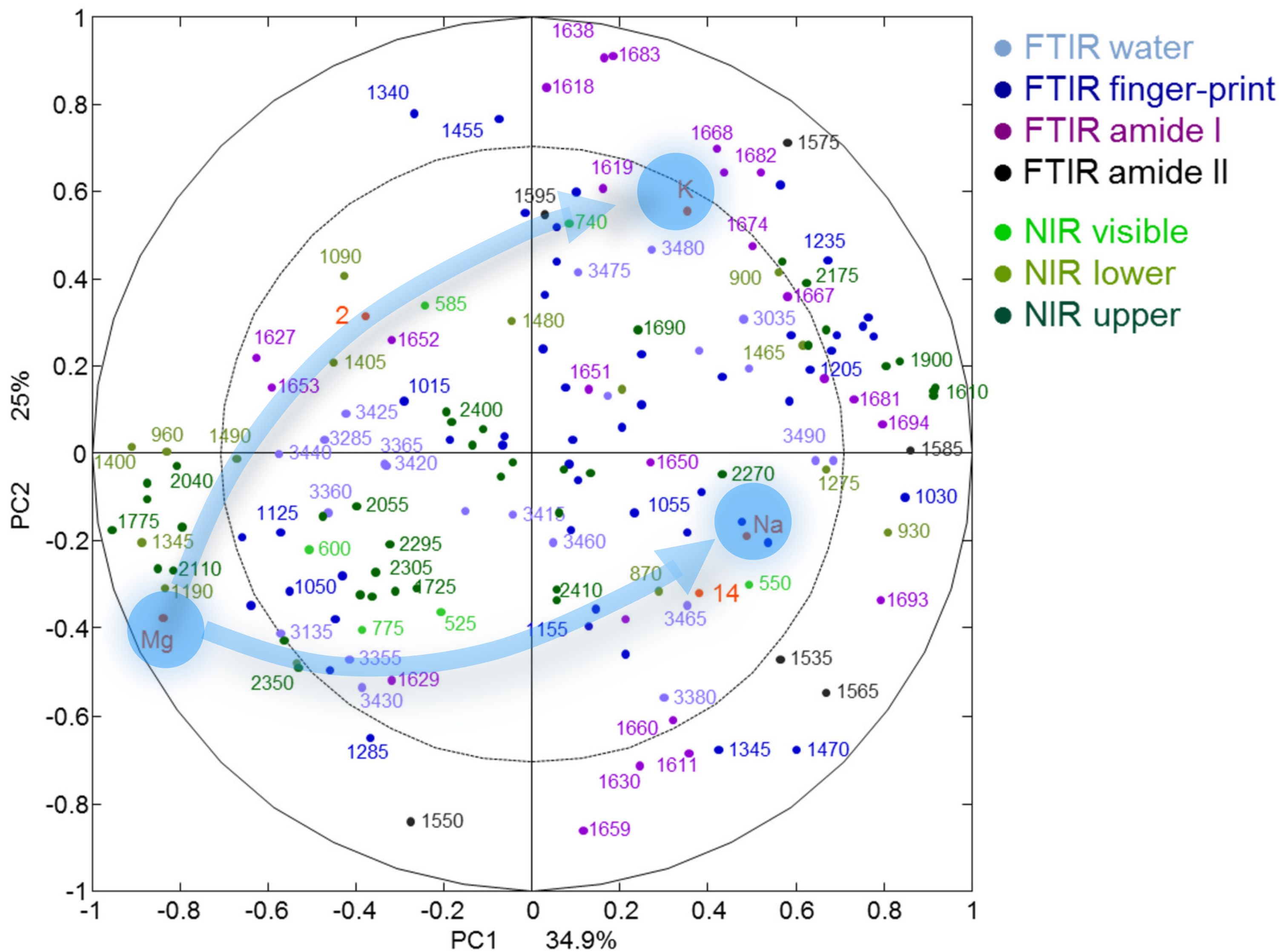
Paper 3
Study overview



Paper 3
spectroscopic analysis



Paper 3
spectroscopic analysis



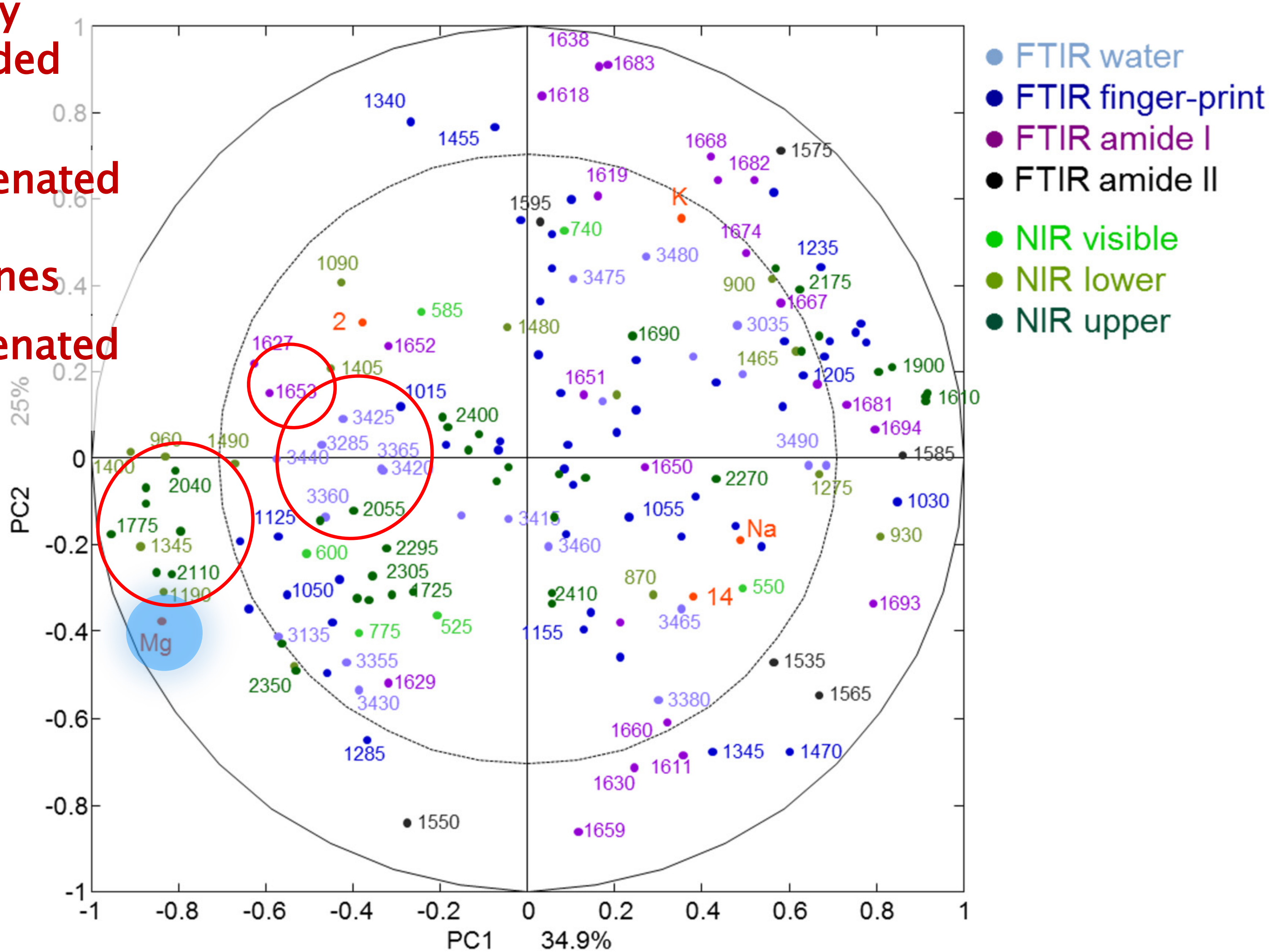
Paper 3

spectroscopic analysis

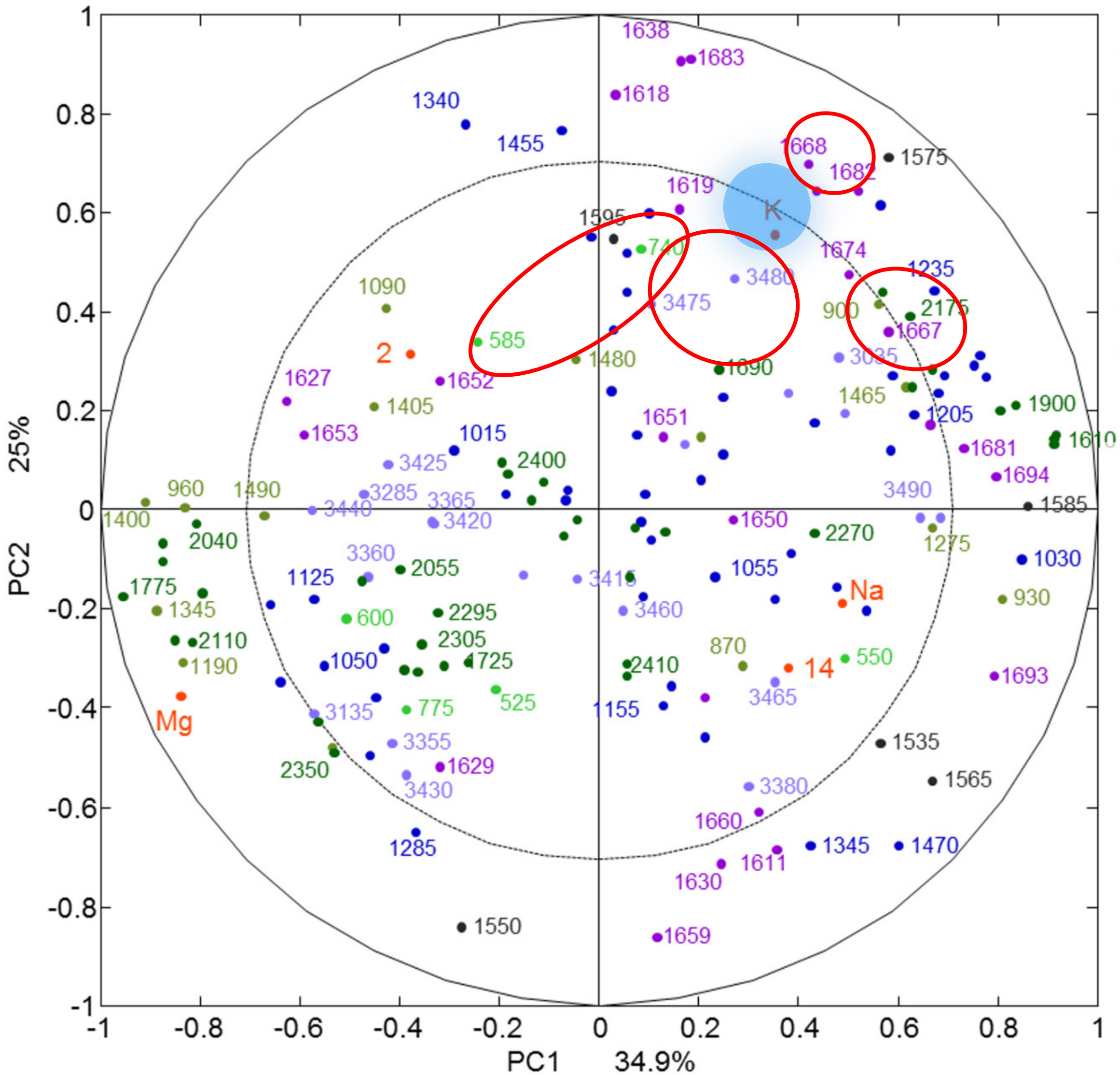
strongly H-bonded water

hydrogenated protein backbones

hydrogenated α-helix



Paper 3
spectroscopic analysis



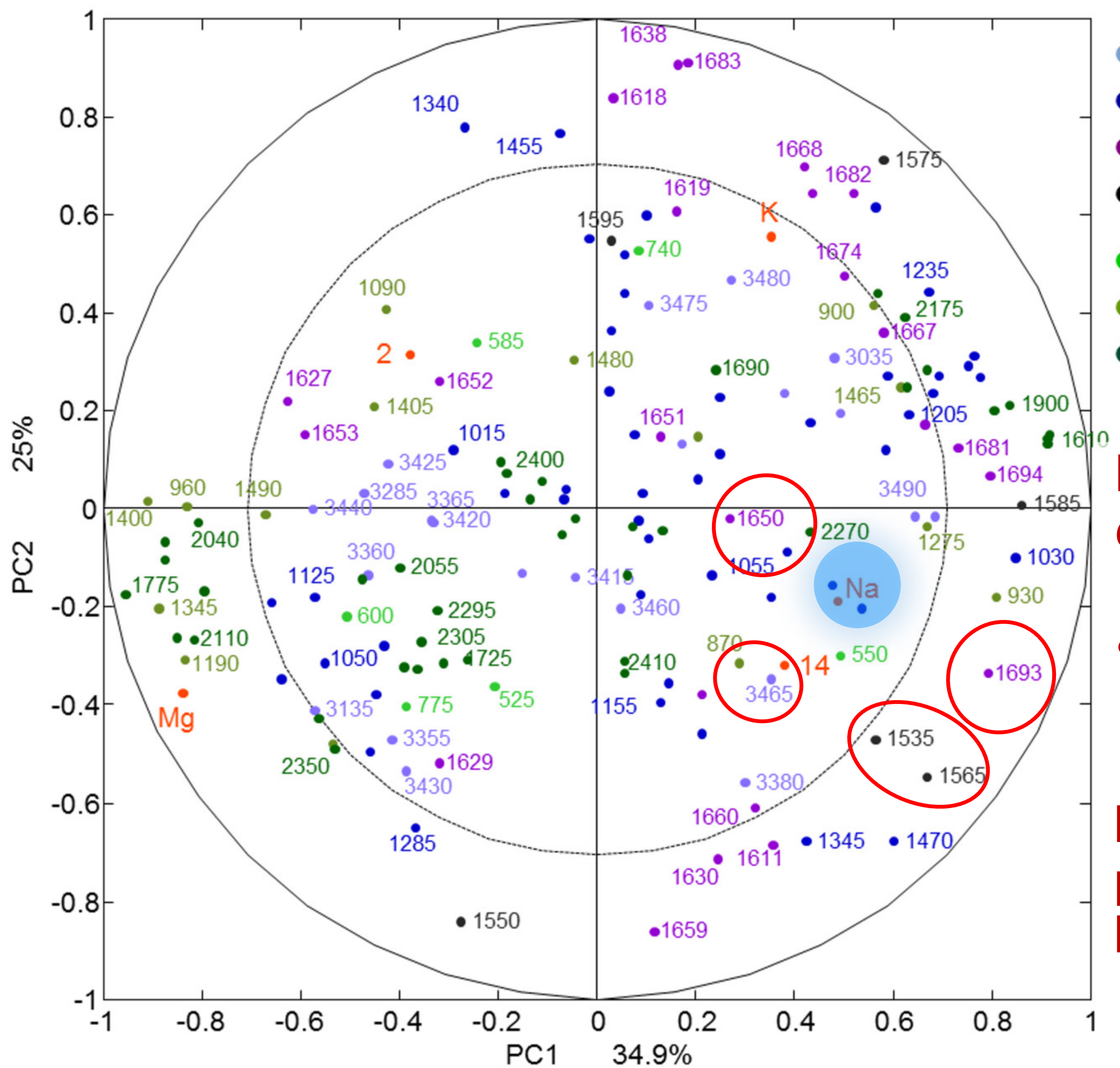
de-hydrogenated α-helix

de-oxi myoglobine

de-hydrogenated protein backbones

Paper 3

spectroscopic analysis



- FTIR water
- FTIR finger-print
- FTIR amide I
- FTIR amide II
- NIR visible
- NIR lower
- NIR upper

little H-bonded α-helix

aggregated β-sheets

hydrogenated protein backbones

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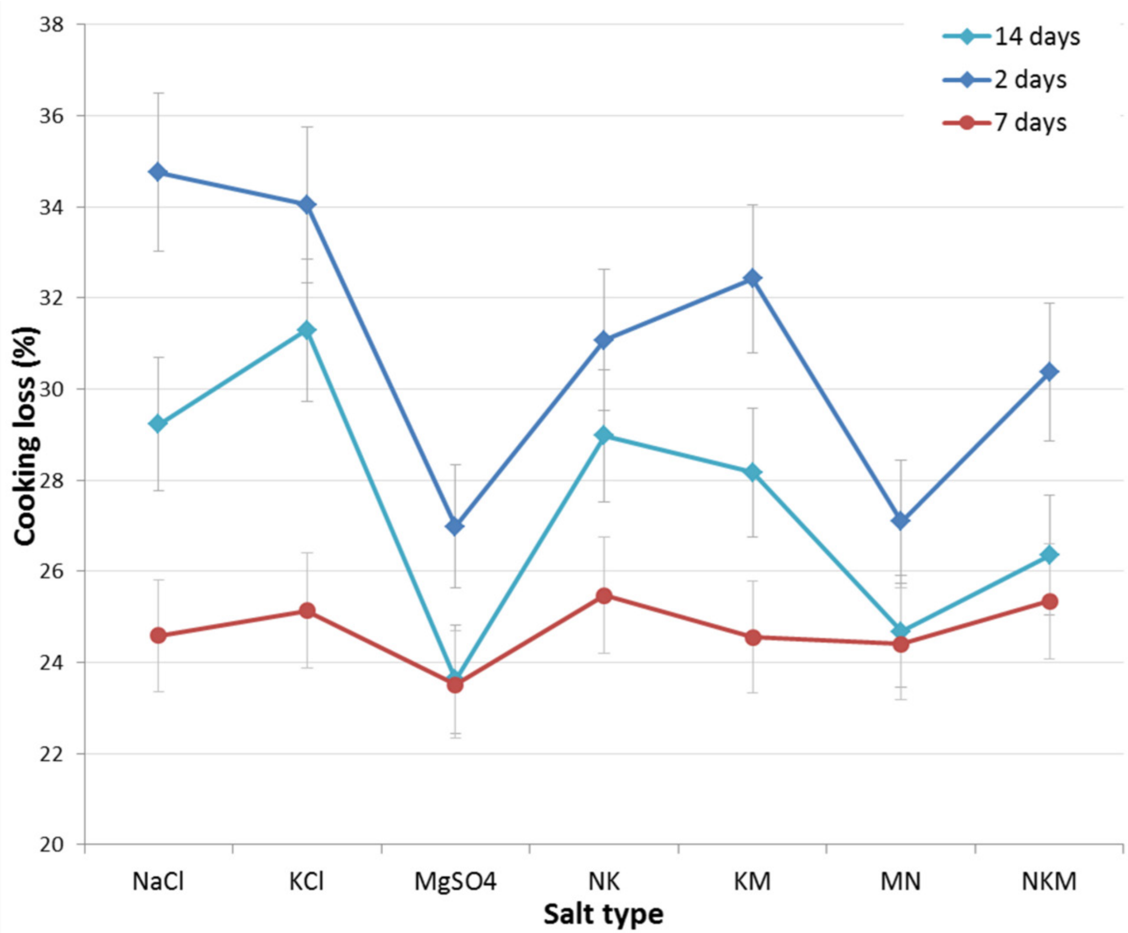
WHC



14 days
2 days
7 days
Cooking loss (%)
38
36
34
32
30
28
26
24
22
20
NaCl
KCl
MgSO4
NK
KM
MN
NKM
Salt type

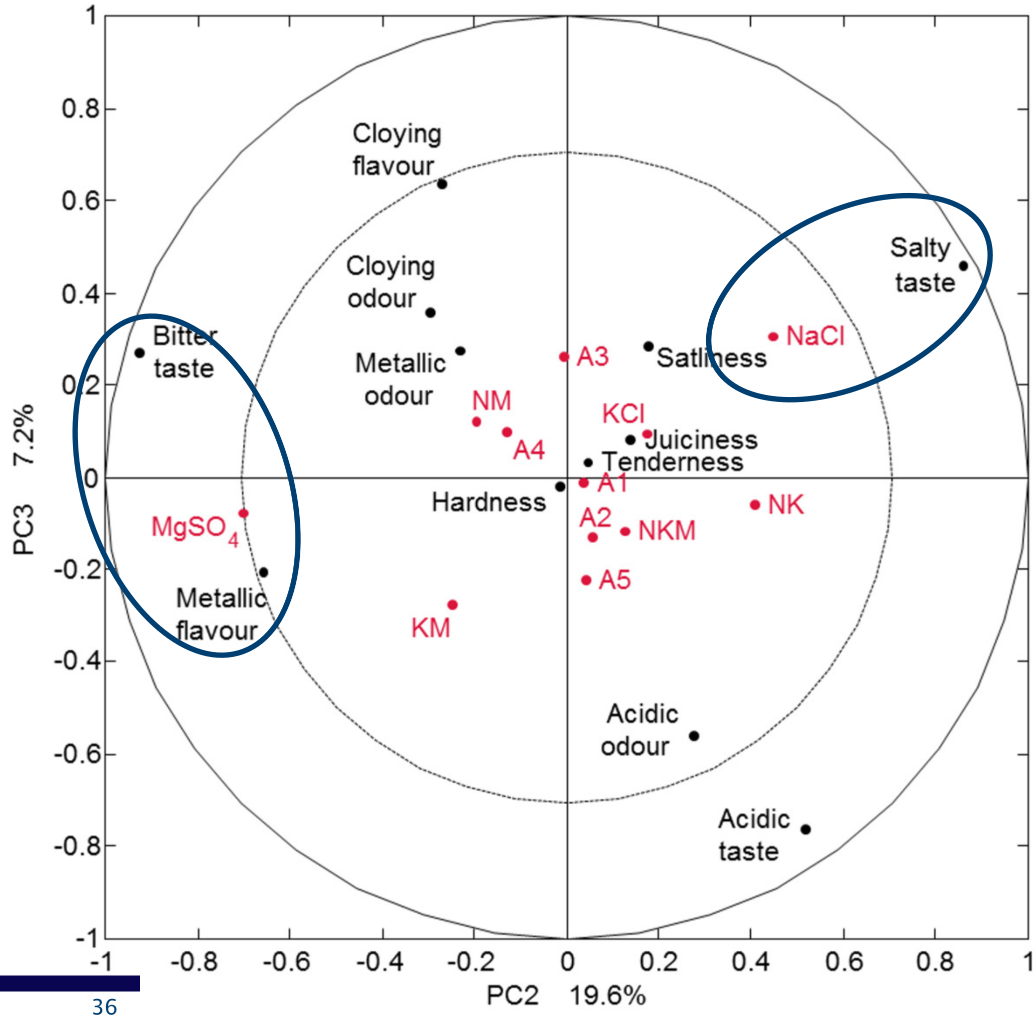
Paper 3
Sensory analysis

sour, salty, bitter, cloyic, metalic

hardness, tenderness, fetness, juiciness

sour, cloyic, metalic

● Evaluated sensory attributes
● Experimental design variables



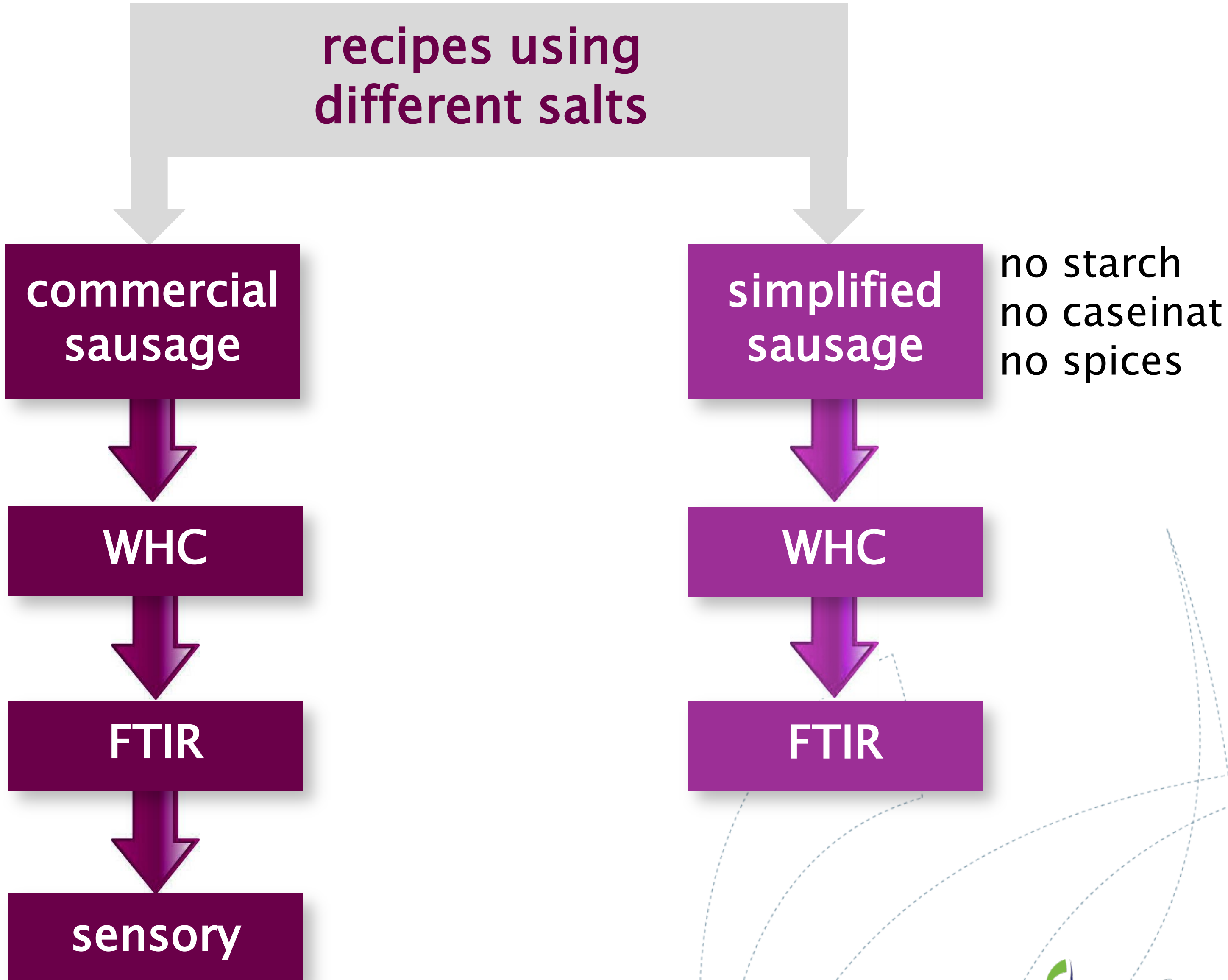
Paper 4

Linking structural changes and sensory properties in meat products subjected to salt reduction and salt substitution: a combined FTIR imaging and sensory study

LWT – Food Science and Technology

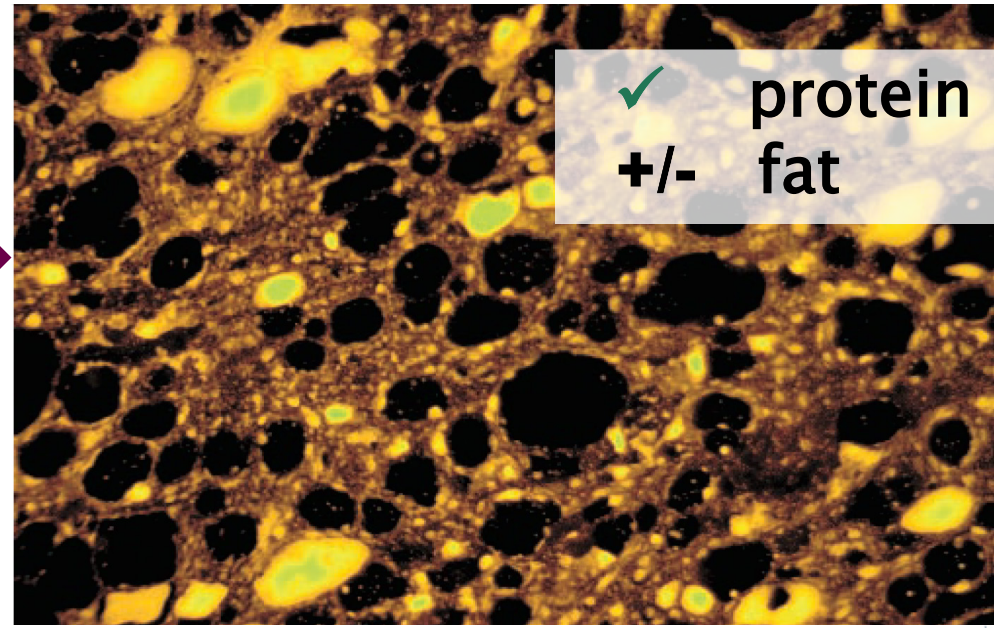
Perisic, N.; Afseth, N. K.; Ofstad, R.; Scheel, J.; Kohler, A.; Linking structural changes and sensory properties in meat products subjected to salt reduction and salt substitution: a combined FTIR imaging and sensory study

Study overview



Paper 4
background

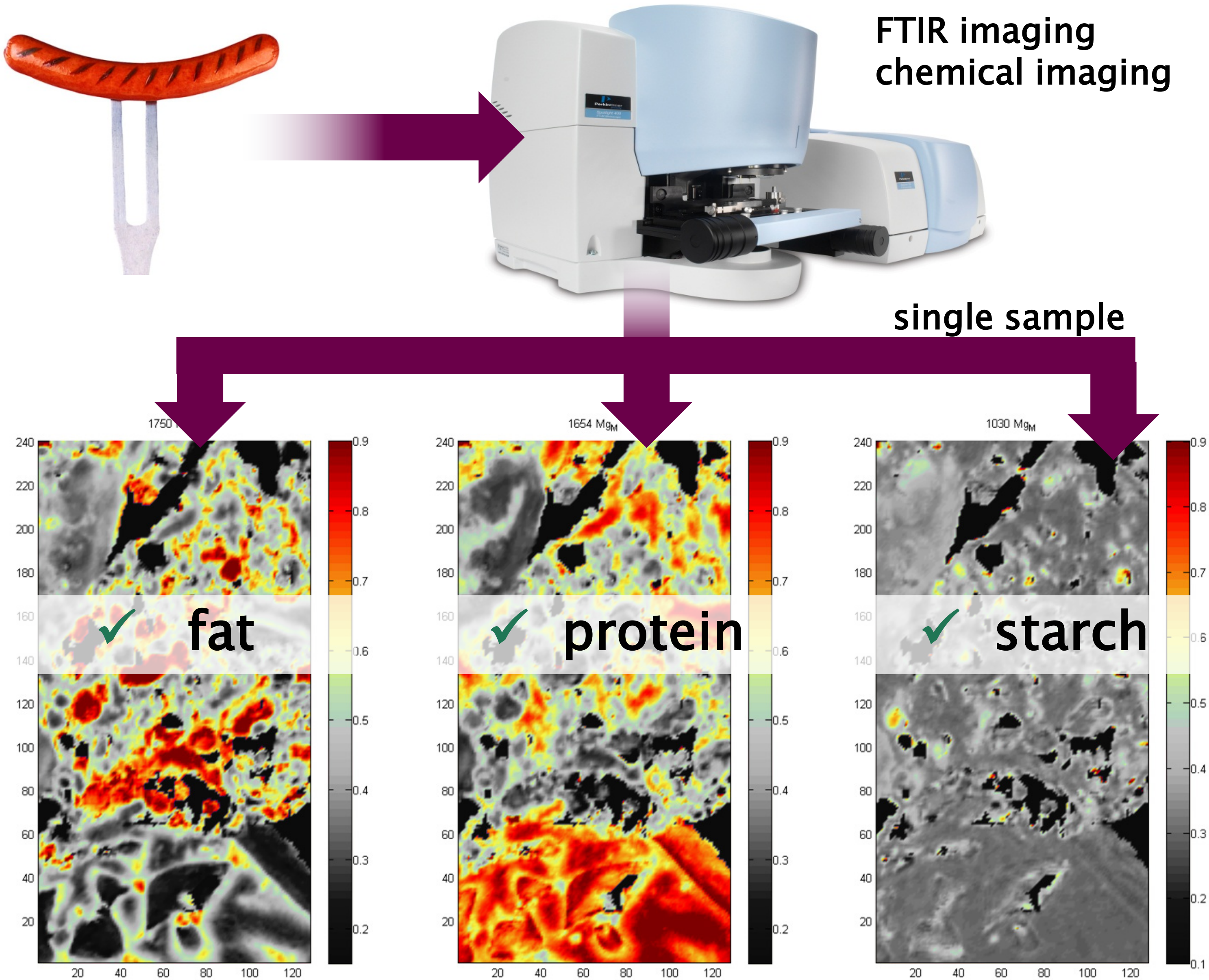
staining



1 sample = 1–few components

Kohler, A.; Høst, V.; Enersen, G.; Ofstad, R., Identification of fat, protein matrix, and water/starch on microscopy images of sausages by a principal component analysis-based segmentation scheme. *Scanning* 2003, *25*, 109–115.

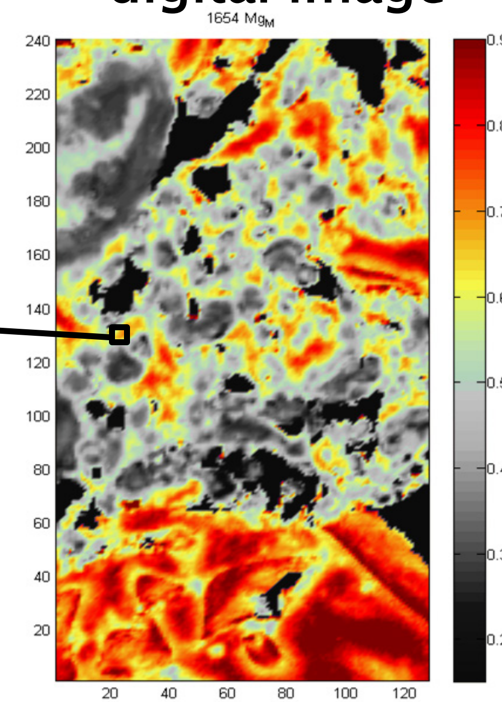
Paper 4
background

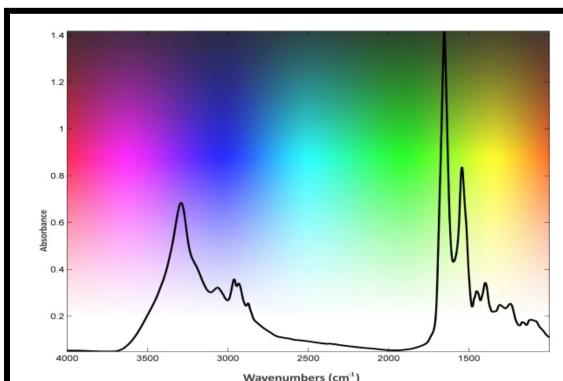


background

single pixel =single frequency

digital image





single pixel =FTIR spectrum
=many frequencies
=many chemical comp.

FTIR image

Paper 4

FTIR imaging results

thin cross
sections

FTIR imaging results

Paper 4

FTIR imaging results

FTIR imaging results

homogeneity

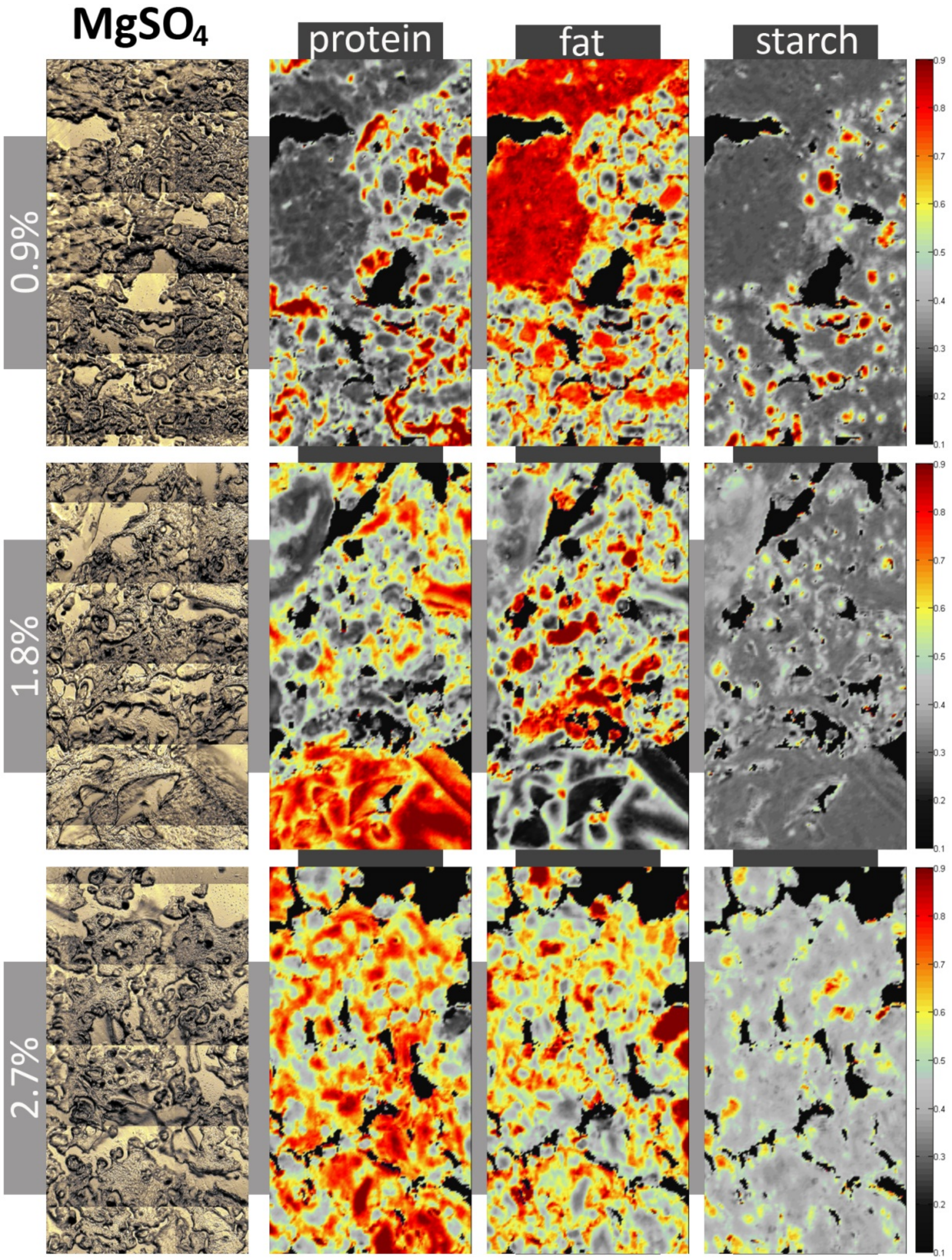
FTIR imaging results

homogeneity

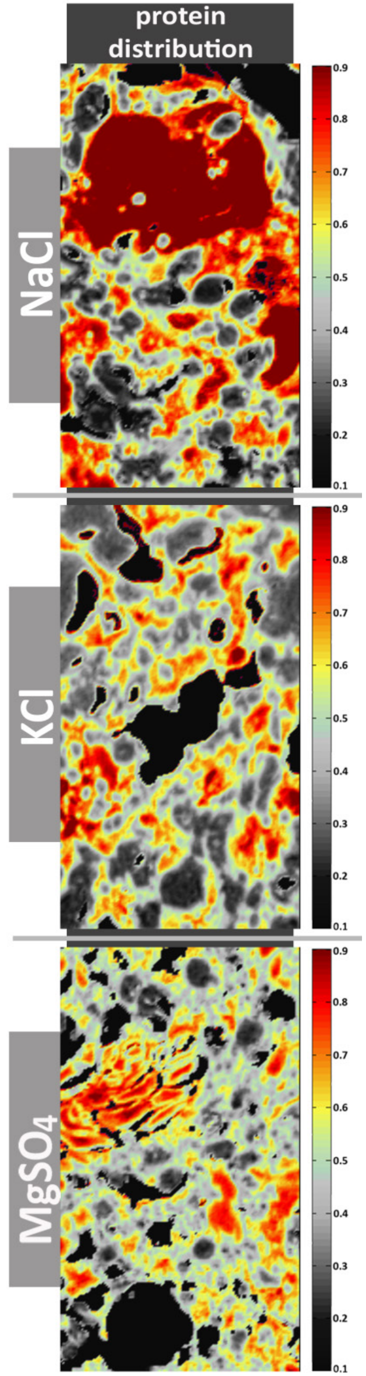
Paper 4

FTIR imaging results

homogeneity

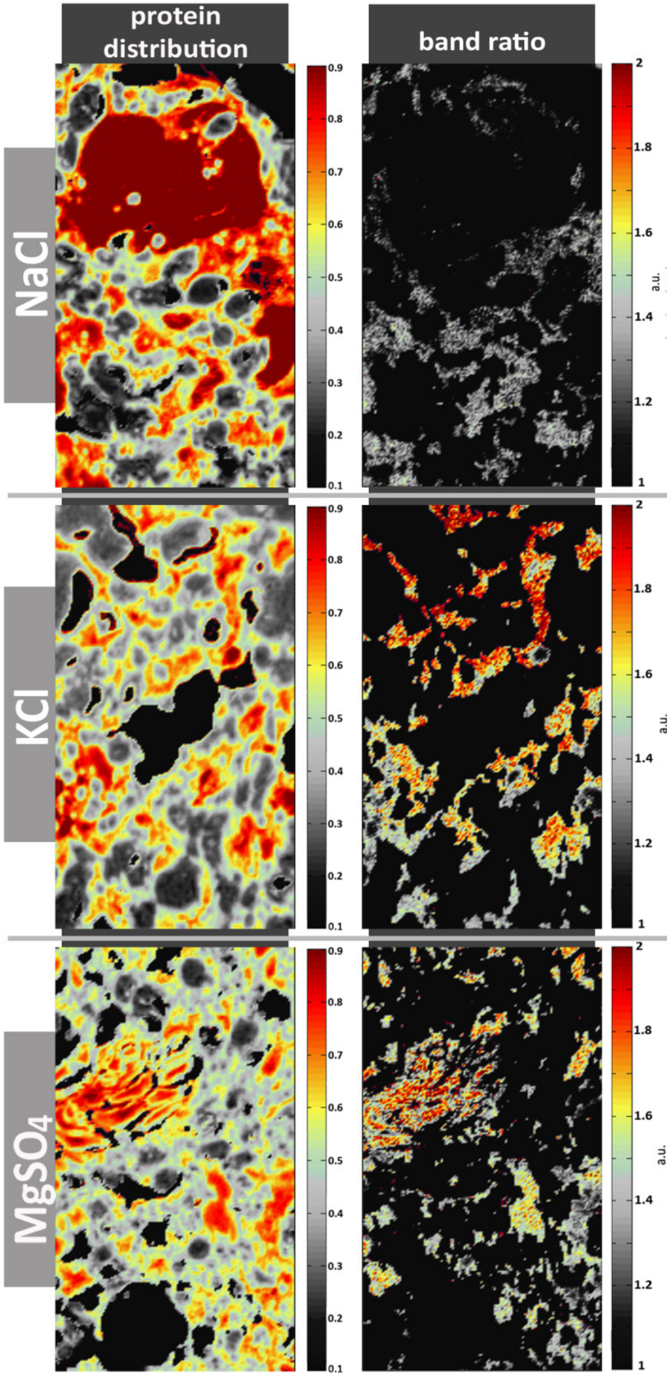


FTIR imaging results

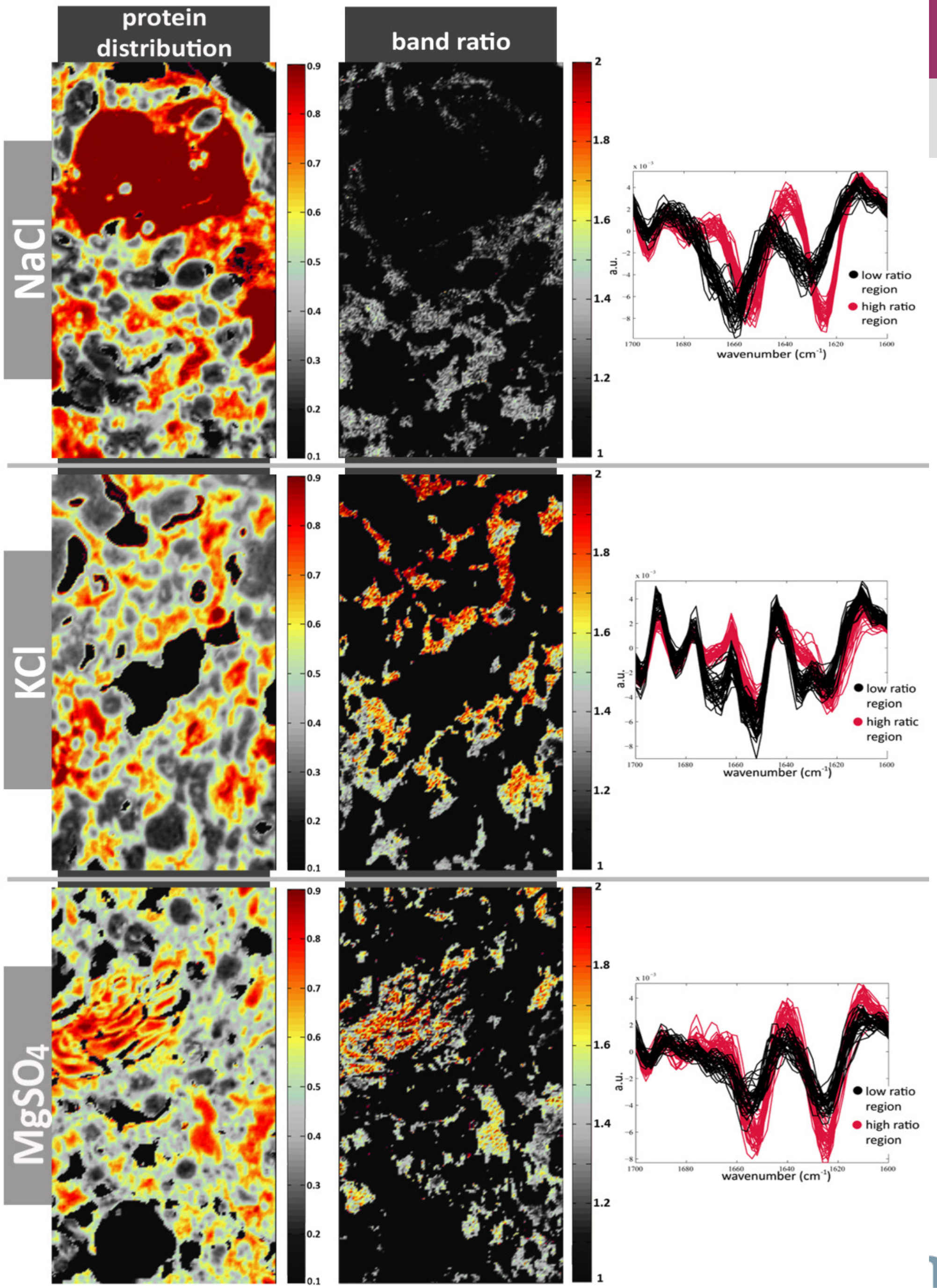


Paper 4

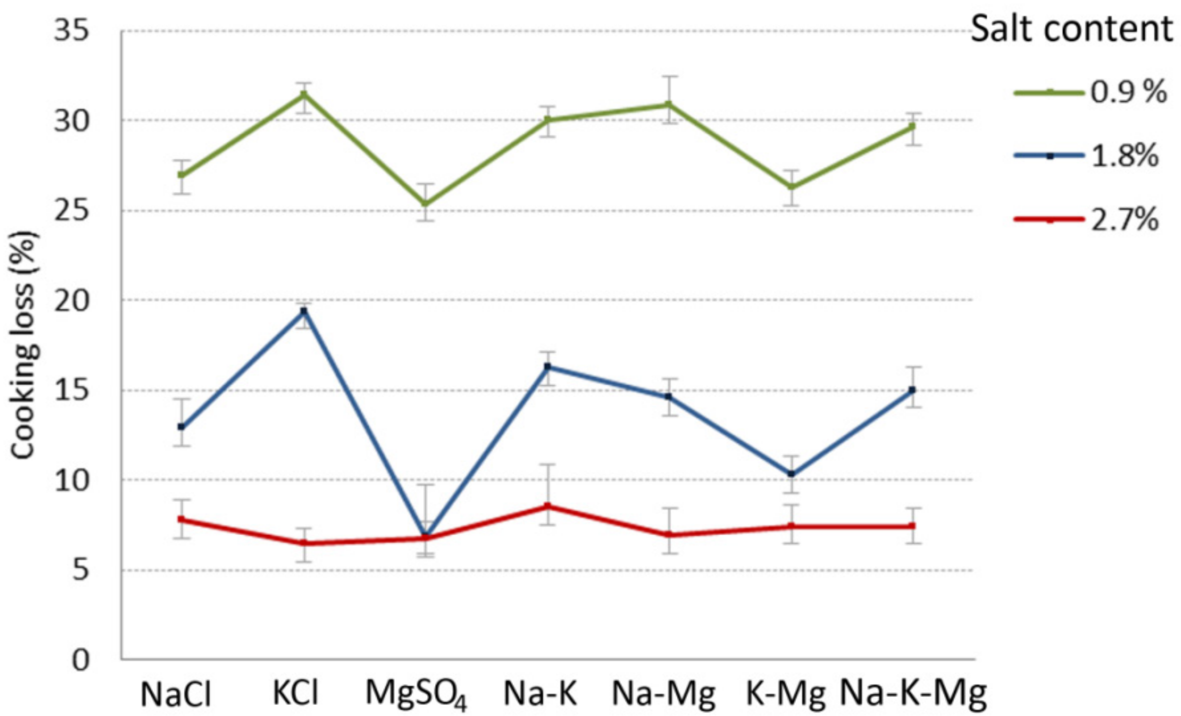
FTIR imaging results



Paper 4
FTIR imaging results



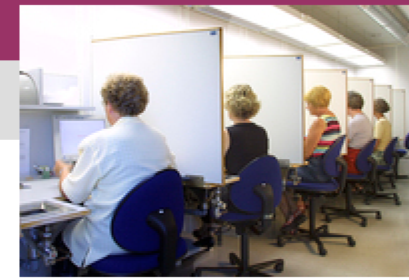
WHC

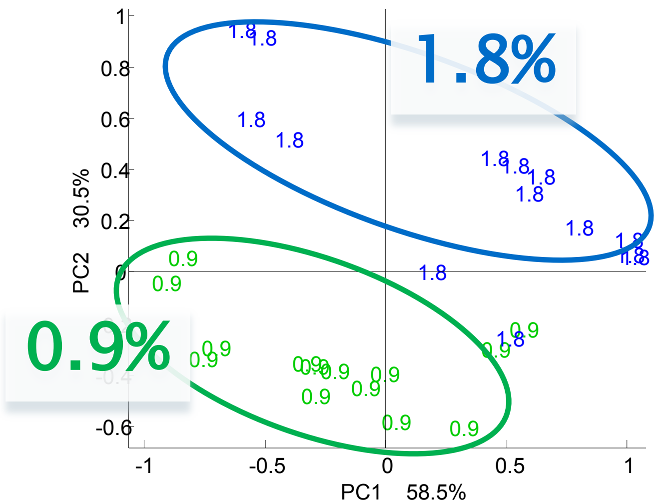


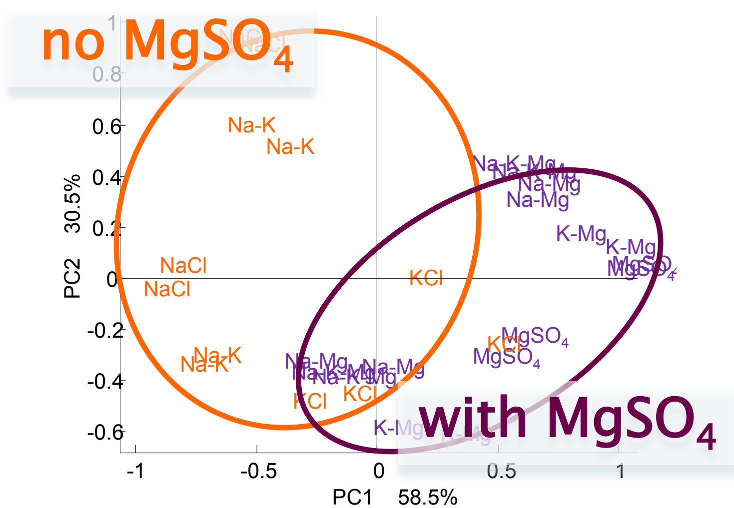
Salt content
0.9 %
1.8%
2.7%
Cooking loss (%)
35
30
25
20
15
10
5
0
NaCl
KCl
MgSO4
Na-K
Na-Mg
K-Mg
Na-K-Mg

Paper 4

sensory







concentration

type

Summary & conclusions

salt concentration > salt type

intact meat – salts alone cause only partial denaturation

minsed+cooked meat – strong denaturation
– large differences between salts

$MgSO_4$ – boldly different effect

$MgSO_4$ – increases WHC
– hydrogenates proteins

KCl – decreases WHC
– de-hydrogenates proteins

} from meat model system to sausages

Summary & conclusions

only affected by concentration – not salt type

higher concentration – desired texture
for all salts

$MgSO_4$ – negative effect on taste
– when used in low portion – acceptable

Summary & conclusions

KCl
- chemically simillar to NaCl
- bad for increasing WHC
- bad for taste (high portions)
- bad for sausage homogeneity

$MgSO_4$
- chemically different NaCl
- good/simillar effect on WHC as NaCl
- good for sausage homogeneity
- **bad** for taste (high portions)

can be used as a substitute to improve WHC / texture / homogeneity

Thank you!