

Total Food 2009

Sustainability of the Agri-Food Chain

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Production of potential bioactive peptides from bovine by-products using commercial enzymes

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General overview

- Use of two enzymes for hydrolysis of low value bovine muscle & offal proteins
 - Validation of hydrolysis methods
 - Initial findings in bioactivity
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Why look for bioactivity?

- Bioactivity - the specific ability or capacity of a compound to achieve a defined biological effect
- Recovery of value from waste by-products
- Meat is potentially rich at bioactive peptides

Bioactive peptides

- Small peptides (2-20 amino acid residues)
- Inactive in parent proteins - released by proteolytic digestion
- Maybe act as regulatory compounds
- Main activities: antihypertensive, antimicrobial, antioxidant, antithrombotic, antiproliferative, opioid, prebiotic

Bioactivities found

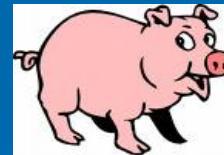
Antihypertensive & opioid activities: egg, milk, cheese, maize, fish

New research shows potential in meat:
...new developing area...

Antihypertensive activity

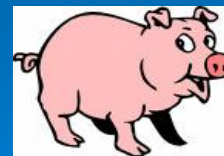


Antioxidant activity



Horns & hoofs

Antimicrobial activity



Spleen & blood

Opioid activity



Blood


Approaches to generate bioactive peptides

- Endogenous (naturally occurring)
- Exogenous (commercial enzymes)

Standardisation of protocols

- Method optimisation
- Reproducibility
- Important for future applications

Aim

- Test the reproducibility of hydrolysis of bovine by-products with commercial enzymes
 - 1-D SDS PAGE & MALDI-TOF/MS
 - Study the antioxidant and antimicrobial bioactivities of the hydrolysates
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- The background of the slide features a pattern of concentric circles resembling water ripples, rendered in a lighter shade of blue against the main blue background.

Purification of bioactive peptides from meat chain

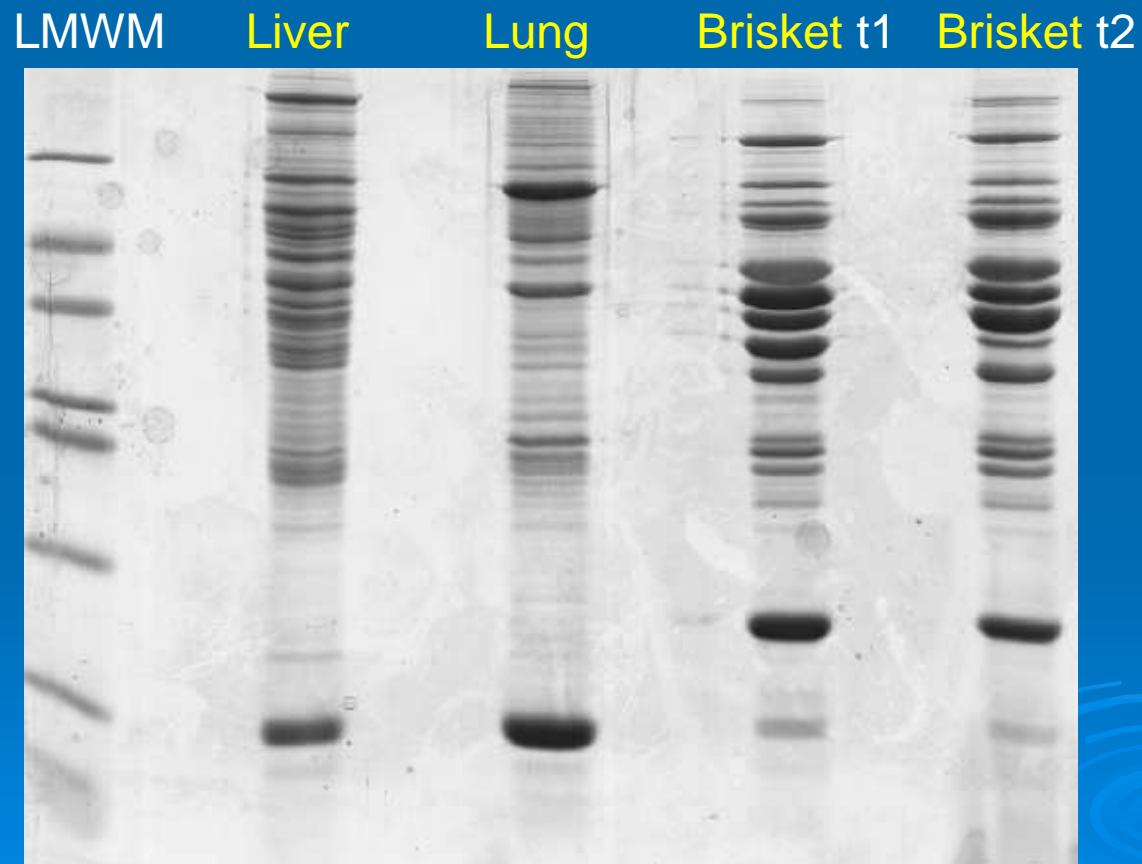
- Organ: Liver
- Smooth muscle: Lung
- Skeletal muscle: Brisket (t_1 , t_2 , two *post-mortem* time points)



Cytoplasmic/sarcoplasmic extracts
(with a low salt buffer)

Characterisation of extracts by 1-D SDS PAGE

Gel electrophoresis: Technique used for the separation of proteins using an electric current applied to a gel matrix



Sample-15ug proteins; 12,5 % resolving gel; 4% stacking gel

Work flow

Hydrolysis of tissue - 2 enzymes - triplicate

Hydrolysis at 7 times: T1 → T7

Profile hydrolysate & check reproducibility
1-D SDS PAGE + MALDI-TOF/MS

Antioxidant activity

Antimicrobial activity

Select most interesting tissue(s)/T

Select most interesting tissue(s)/T

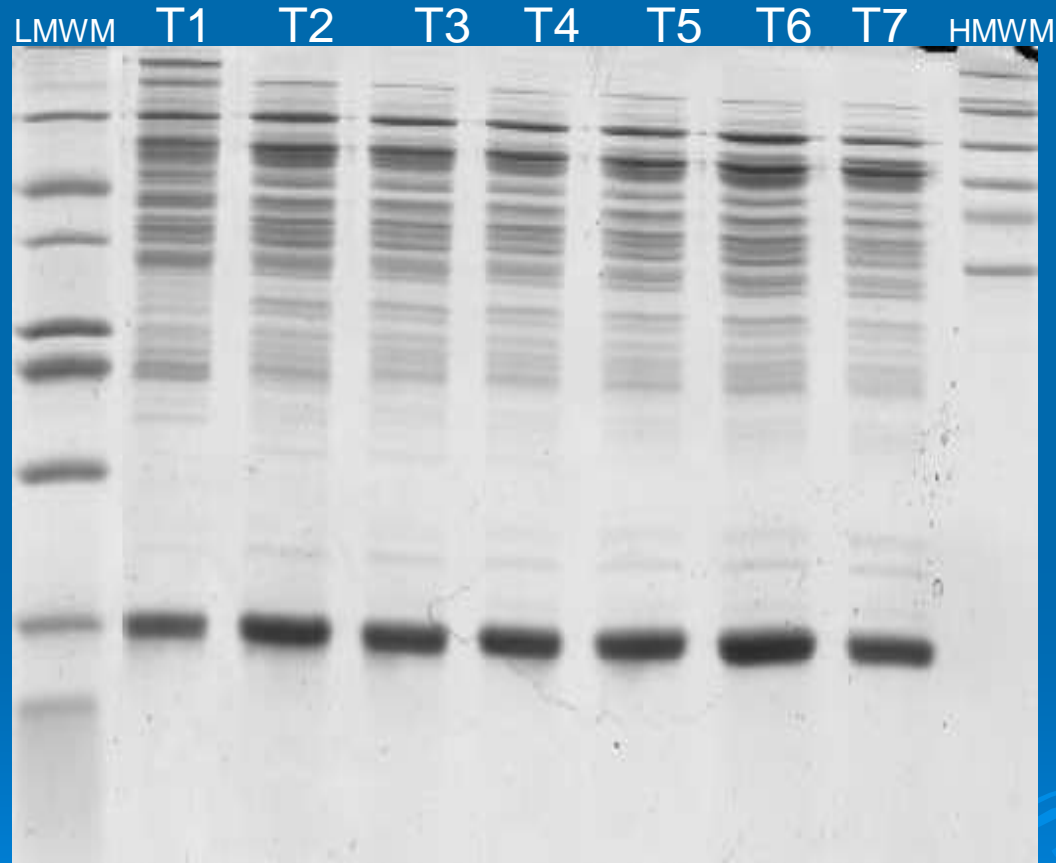
Purify/characterise antioxidant peptides

Purify/characterise antimicrobial peptides

1-D SDS PAGE

Study the high molecular weight proteins

Increased hydrolysis →



Liver
cytoplasmic
proteins:
Enzyme 1

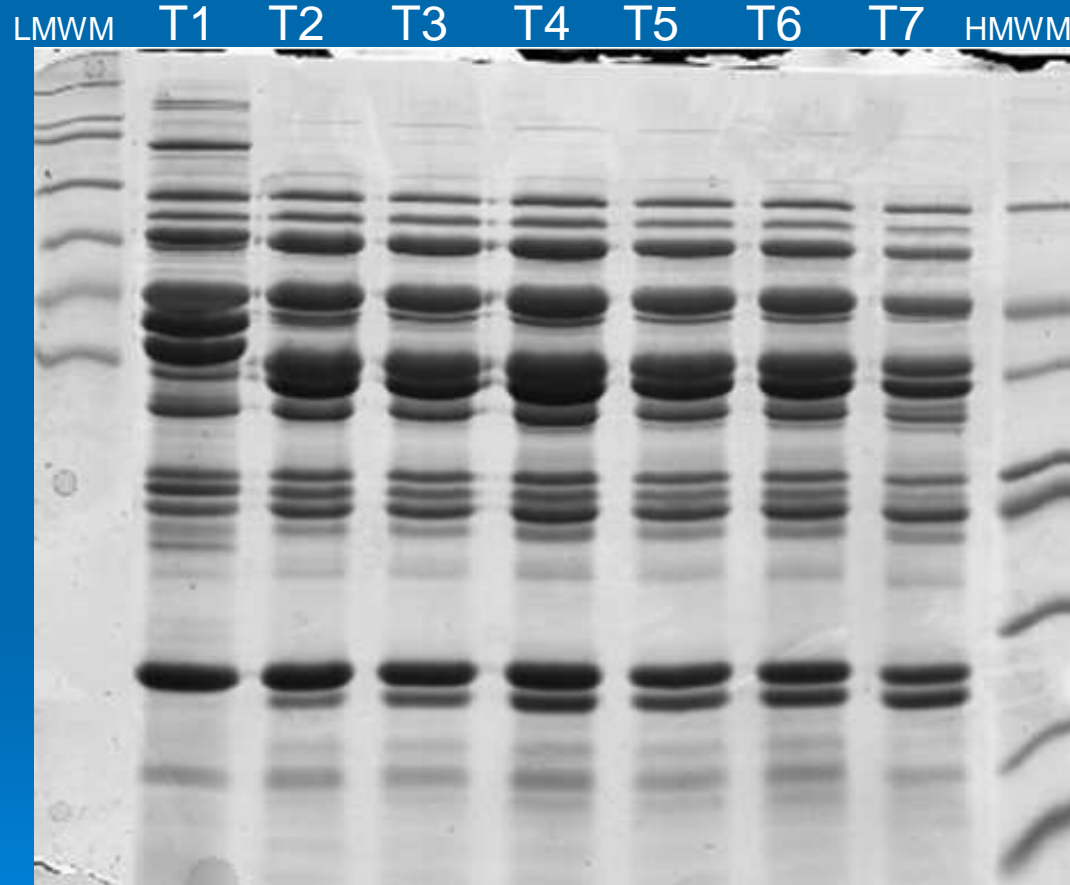
LMWM: 66-6.5KDa
HMWM: 205-36KDa

Sample-15ug proteins; 15 % resolving gel; 4% stacking gel

1-D SDS PAGE

Study the high molecular weight proteins

Increased hydrolysis →



Brisket t2
sarcoplasmic
proteins:
Enzyme 2

LMWM: 66-6.5KDa
HMWM: 205-36KDa

Sample-15ug proteins; 12,5 % resolving gel; 4% stacking gel

MALDI-TOF Mass Spectrometry

Study the low molecular weight proteins

Mass Spectrometry: determination of the elemental composition of a sample or molecule

Principle: ionizing chemical compounds to generate charged molecule or molecule and measurement of their mass-to-charge ratios (from the motion of the ions as they transit through electromagnetic fields)

Liver, Lung, Brisket t1, Brisket t2:
Hydrolysed with enzyme 1 & 2: T1 & T4 in triplicate
Ultrafiltrated 0-10KDa

Results:

- ✓ peptides profile observed for all the tissue types using both enzymes at 2 time points
- ✓ reproducibility of peptides production observed

Bioactivity

- **Antioxidant activity observed:** DPPH assay
Liver, Lung, Brisket t1 hydrolysed fractions showed activity at several hydrolysis time points
(Liver highest, Brisket t1 lowest)
- **Preliminary antimicrobial activity observed:**
Liver - inhibition towards *E.coli* (60-100%)

Overall conclusions

- Reproducibility of hydrolysis of bovine Liver, Lung, Brisket t1 & t2 with 2 enzymes by 1-D SDS PAGE and MALDI-TOF/MS
- Antioxidant activity found in hydrolysis fractions of Liver, Lung, Brisket t1
- Antimicrobial activity found in hydrolysis fractions of Liver

Future work

- Assess antioxidant activity in Brisket t2
- Assess antimicrobial activity in all tissue types
- Study the most interesting hydrolysis fractions from selected tissue types using fractionation with RP-HPLC

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Thank you
for your attention!...

