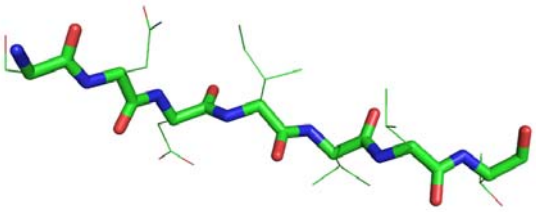
Project number: 5636
Funding source: DAFF (06RDTAFRC472)
Date: March 2012
Project dates: Dec 2006 – Nov 2010

Nutraceutical and functional food bioactive peptides in beef, bovine offals and fermented meat products



Key external stakeholders:
Beef processing sector

Practical implications for stakeholders:
The main outcome of this research provides support for a strategic approach to recovering value from the meat processing chain. Clear evidence has been presented that bioactive peptides can be generated from low value meat and offal. The capabilities for generating, isolating and characterising bioactive peptides from meat sources have been established at Teagasc. The assays have been optimized and are now part of a full peptide isolation, purification and characterization infrastructure available to the Irish food industry. The potential of generating bioactive peptides from bovine offal and low value muscle has been demonstrated in this project. Research in the extraction of commercially valuable peptides from meat and meat industry by-products is in its infancy and this project provides a solid foundation on which future development and discovery will inevitably yield scientific advancement and commercial return.

Main results:
- Capabilities established for generation, isolation and characterization of bioactive peptides from meat sources.
- Antioxidant peptides successfully generated from bovine liver.
- Peptides with antioxidant and antihypertensive activity isolated from brisket fractions.
- Peptides generated from bovine lung which exhibited antioxidant, antihypertensive and antithrombotic activity.
- Heart peptide fractions displayed antioxidant and antimicrobial activity.
- Bioactive peptides generated from proteins isolated from bovine muscle.

Opportunity / Benefit:
Knowledge generated in this research will be beneficial in developing strategies to recover value from meat processing streams. The scientific expertise and infrastructure developed in this project will act as a spring board to encourage the exploitation of the protein component of offal and waste streams produced by the meat industry as a source of high value biologically active ingredients with food and pharmaceutical applications.

Collaborating Institutions:
UCC

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1. Project background:

The 2011 data from Bord Bia place the value of Irish beef exports to the Irish economy as €1.5 billion, accounting for 25% of Ireland's food and drink exports. A significant amount of secondary, by-product and waste material is generated through beef processing systems. Calculated on a per head basis the quantity of reported waste material averaged at 263kg/head in 2008 (Sustainable Practices in Irish Beef Processing, Enterprise Ireland, 2008). Much of this material can be considered as a rich source of functional proteins and peptides which exhibit a diversity of functional activity. While some products such as offal have markets, these tend to be lower value and also have an associated carbon footprint (e.g. Asian markets). Generating bioactive peptides with applications in food and non-food (pharmaceuticals etc) sectors holds much potential for accessing higher value markets.

2. Questions addressed by the project:

- What methods need to be established to generate peptides from bovine offals (lung, liver, heart) and low value muscle (brisket) with a view to screening for bioactivity?
- Can we establish bioassays to detect and measure bioactivity: antioxidant, antihypertensive, antithrombotic and antimicrobial?
- Do peptide fractions generated from bovine offal & muscle exhibit bioactivity?
- How do these peptides react in a meat product?

3. The experimental studies:

- A number of methods for peptide generation from muscle, liver, lung and heart were investigated.
- Bioassays were established to monitor activity in extracts from the various tissue sources.
- Bioactivity was analysed in a number of peptide rich fractions.
- Peptide fractions were characterized by mass spectrometry and mass spectral data was analysed using appropriate software programmes.
- An initial trial was carried out to include antioxidant peptides in burgers.

4. Main results:

Peptide generation and bioassays:

A number of methods were developed and/or optimized during the course of this project which are valuable for the assessment of bioactivity in bovine offal and low value muscle. Initially methods focused on protein extraction, peptide generation and characterisation. **Antioxidant activity** was measured in a number of ways: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, the ferric reducing activity power (FRAP) assay and the Fe2+ chelating activity assay). **Antihypertensive assay:** a fast and cost effective assay for the detection of ACE inhibitory peptides was established. **Antithrombotic / Atherosclerosis prevention**: a PAF (platelet activating factor) Acetylhydrolylase inhibitor screening assay was developed and validated for detecting antithrombotic activity. Plasma PAF-AH has been linked to atherosclerosis and may be a positive risk factor for coronary heart disease hence. This assay is very useful for high throughput screening of a large number of samples. **Antimicrobial activity** was determined using the agar well diffusion assay was against a range of bacterial pathogens including *Enterobacter (Cronobacter) sakazakii*, *Salmonella* sp., *Listeria monocytogenes*, *Streptococcus* spp. and *Escherichia coli* O157.

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Assessing bioactivity in bovine offal and low value muscle

Liver: Following hydrolysis (thermolysin) and ultrafiltration (10-kDa and 3-kDa molecular weight cut off) of bovine liver sarcoplasmic proteins, filtrates and RP-HPLC fractions were assessed for antioxidant activity. The peptide contents of each fraction were characterised using electrospray quadrupole time-of-flight (ESI-Q-TOF) mass spectrometry with the resultant spectrum analysed using the software programmes Protein Lynx Global Server 2.4 and TurboSEQUEST. Similarities between the amino acid composition of characterised peptides and previously reported antioxidant peptides were found demonstrating that liver can be utilised as raw material for the generation of bioactive peptides.

Brisket: Sarcoplasmic proteins isolated from the bovine brisket (*Pectoralis profundus*), were hydrolysed with papain for 24 hrs at 37 °C and ultra-filtrated (10-kDa and 3-kDa). Hydrolysates and filtrates displayed both antioxidant and antihypertensive activity. The peptidic contents were analysed using an ORBITRAP mass spectrometer, and mass spectral data obtained was analysed using TurboSEQUEST. Similarities observed between the amino acid sequences of the peptides identified in this study and previously identified bioactive peptides were outlined.

Lung: Bovine lung tissue was hydrolysed (thermolysin for 8 hours at 37 ˚C) and filtrated (10-kDa, 3kDa). Resultant filtrates were tested for antioxidant, antihypertensive and antithrombotic activities. Both filtrates showed antioxidant activity while the 10kDa filtrate displayed ACE-I inhibitory activity. No PAF-AH inhibitory activity was detected. Peptides isolated from the filtrates were identified using electrospray quadrupole time-of-flight (ESIQ-TOF) mass spectrometry. Homologies between the sequences of three identified peptides and previously reported ACE-I inhibitory peptides were observed

Heart: Both fermentation with lactic acid bacteria and proteolysis (pronase E, proteinase-K, ficin, papain & trypsin) were examined for production of bioactive peptides from bovine heart. The use of proteolytic enzymes proved to be a more effective strategy for producing peptides from bovine heart proteins than the use of lactic acid bacteria. Heart sacroplasmic proteins hydrolysed by Pronase E and fractionated on HPLC were assessed for antioxidant (DPPH) activity. One fraction, in which 4 peptides were characterized, exhibited strong antioxidant activity. Hydrolysates from heart myofibrillar proteins showed little potential to be used as antioxidants or antimicrobial agents.

Myofibrillar proteins actin and myosin were extracted from skeletal muscle and hydrolysates (pepsin, trypsin and α-chymotrypsin) screened for antimicrobial, antioxidant, antithrombotic and ACE inhibitory activities. ACE-inhibitory activity was detected in the hydrolysates: chemical synthesis of peptides confirmed activity. Pepsin hydrolysates exhibited highest antioxidant activity. No antimicrobial or antithrombotic activity was detected.

Preliminary study to incorporate bioactive peptides into meat product

Hydrolysates were obtained following thermolysin digestion (2 hours at 37°C) of bovine liver. Cytoplasmic proteins were freeze dried and incorporated into beef patties (0, 0.5 or 1.0% w/w). These were modified atmosphere packaged (80% O_2:20% CO_2) and stored at 4°C for 10 days during which time the colour and the lipid stability were monitored. No delay in lipid oxidation or improvement in colour stability was observed. The influence of the product matrix on the antioxidant activity needs to be investigated to determine optimal concentration etc.: this was a preliminary study and hence further analysis was outside the scope of the project.

5. Opportunity/Benefit:

This research has successfully provided the evidence that bioactive peptides can be generated from low value meat processing products. Properties of these bioactive co-products go beyond their value as nutritive proteins/peptides. Data generated is of high relevance to the meat processing sector as lays the foundation for innovative approaches to accessing higher value markets and ensuring the long term survival and continued growth of the sector. Further validation of these peptides will be required e.g. through continued chemical synthesis of the peptide sequences. Additionally further investigation of the meat matrix with the peptides and/or protection of the peptides will help ensure peptides remain active in the product. Outputs of this research are also of high relevance to the scientific community as evidenced by the peer reviewed publications arising from this research.

6. Dissemination:

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Dissemination has taken the form of peer-reviewed publications, technical publications, conference presentations, workshops and one-to-one discussions with industry. These discussions are continuing beyond this project into the development of strategies to extract or recover value from beef waste streams.

Main publications:

Di Bernardini R, Rai DK, Bolton D, Kerry J, O'Neill E, Mullen AM, Harnedy P, Hayes M.(2011). Isolation, purification and characterisation of antioxidant peptidic fractions from bovine liver sarcoplasmic protein thermolysin hydrolyzate. *Peptides* 32: 388-400

Di Bernardini, R.,, Bolton, D., Harnedy, P., Kerry, J., O'Neill, E., Mullen, A.M. and Hayes, M. (2011). Antioxidant and Antimicrobial Peptidic Hydrolysates from Muscle Protein Sources and By-products. *Food Chemistry* 124 (44): 1296-1307

Di Bernardini, R., Mullen, A.M., Bolton, D., Kerry, J., O'Neill, E., and Hayes, M. (2011)
Assessment of the angiotensin-I-converting enzyme (ACE-I) inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain and characterisation of associated bioactive peptidic fractions. *Meat Science*, Volume 90 (1): 226-235

Popular publications:

Mullen, A.M., (2011) Meat - more than just a steak. *Teagasc Food Innovator* Issue 10 Spring 2011

Mullen, A.M., (2009) Extracting value and health benefits from beef. *Ashtown Food Innovator*, Issue 4 spring 2009

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