

**Project number:** 5984  
**Funding source:** Teagasc

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**Project dates:** Sept 2008 – Aug 2011.

## Investigation of Bioactive Peptides in Food through the application of Mass Spectrometry



### Key external stakeholders:

Meat sector, Nutraceutical Industry and Dairy.

### Practical implications for stakeholders:

- The analysis of bioactive peptides is difficult due to the complex nature of food samples and the requirement for specialised analytical instrumentation.
- In this research, methodology and systems were developed by Teagasc researchers for the analysis of bioactive peptides using quadrupole time of flight mass spectrometry.
- A facility and expertise is now available to support the food industry and collaborative research.

### Main results:

- Analytical systems were developed at Teagasc for the measurement of bioactive peptides in food samples using nano—liquid chromatography coupled to high resolution mass spectrometry.
- A method was developed for the determination of peptide markers for different animal species.
- The system was successfully applied to identify and characterise a range of bioactive peptides in meat.

### Opportunity / Benefit:

A bioanalytical facility is available at Teagasc for the analysis of bioactive peptides in food products. This can be utilised by the food industry to identify new bioactives and support product development.

### Collaborating Institutions:

Cork Institute of Technology

**Teagasc project team:** Dr. Martin Danaher (PI)  
Dr. Dilip Rai

**External collaborators:** Dr Ambrose Furey, Cork Institute of Technology

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### 1. Project background:

The objective of the project was to establish applications for the detection and characterization of small molecules, peptides and proteins molecules using liquid chromatography coupled to high resolution mass spectrometry (nano-LC-QToF-MS). This work was facilitated through the application of a nano-LC-QToF-MS that was acquired through FIRM strategic equipment budget administered by the Department of Agriculture, Food and the Marine. This instrument combines the capabilities of Quadrupole and TOF analysers to provide high confidence in structural identification compared to other low resolution instruments. The use of nano-liquid chromatography allows small volumes of samples to be analysed e.g. peptide analysis in complex mixtures.

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### 2. Questions addressed by the project:

Can methodology be developed to identify peptides and other compounds in food?  
Is the technology suitable for application in routine analysis?

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### 3. The experimental studies:

- Methods were set-up for the analysis of peptides in different tissue extracts.
- A LC-Qtof method and sample preparation procedure was developed to detect meat contamination with foreign peptides.
- Methods were developed to detect the adulteration of health food products using non-targeted approaches.
- The methods developed in these studies were evaluated using real samples and through inter-laboratory studies.

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### 4. Main results:

#### Methodology for identification of bioactive peptides

Methodology was developed for the identification of peptides in protein extracts using electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) mass spectrometry and data was processed using different databases including Protein Lynx Global Server 2.4 and PEAKS<sup>®</sup> as well Mascot server.

#### Test for Chicken adulteration

A method was developed and established for the detection of gelatin from different species in the dripping of thawed chicken muscle. Juice drippings were collected and purified using molecular weight cut-off devices and solid phase extraction. Following tryptic digestion samples were analyzed and characterized using LC-QToF. The method was successfully evaluated through participation in an inter-laboratory ring trial organized by the Food and Environmental Research Agency in York.

#### Analysis of Health Food Adulteration

A non-targeted approach method was established for the identification of adulterants in food supplements using LC-Qtof. Samples were extracted with acetonitrile, concentrated and filtered prior to LC-Qtof analysis. Sample extracts were subsequently analyzed using a targeted method on a triple quadrupole LC-MS/MS system. The method was successfully applied to identify the contamination of products with synthetic chemicals.

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### 5. Opportunity/Benefit:

A bioanalytical facility is available at Teagasc for the analysis of bioactive peptides in food products. This can be utilised by the food industry to identify new bioactives and support product development. Non-targeted methodologies developed on the project can be utilised to characterise adulteration of food products.

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### 6. Dissemination:

Research from this project was dissemination through conferences, workshops and peer reviewed publications. Dissemination will continue after the end of this project because the technology is now being applied on other projects.

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**Main publications:**

Di Bernardini, R., Rai, D. K., Bolton, D., Kerry, J., O'Neill, E., Mullen, A. M., Harnedy, P., & Hayes, M. (2011). 'Isolation, purification and characterization of antioxidant peptidic fractions from a bovine liver sarcoplasmic protein thermolysin hydrolyzate' *Peptides* 32(2), 388-400.

Moran, S., Robertson, K., Paradisi, F., Rai, D. K., & Murphy, C. D. (2010). 'Production of lipopeptides in *Bacillus* sp. CS93 isolated from Pozzol.' *FEMS microbiology letters* 304(1), 69-73.

Gadaj, A., Rai, D.K., Furey, A., Danaher, M. (2011) 5th International Symposium on Recent Advances in Food Analysis, RAFA 2011, Prague, 1-4 November 2011; Martin Danaher Application of UPLC-MS/MS for Determination of Synthetic Adulterants in Slimming Food Supplements (poster presentation).

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**7. Compiled by:** Dr Martin Danaher

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