# ADVANCED CHARACTERISATION AND EVALUATION OF MEAT HYDROLYSATES AS FUNCTIONAL INGREDIENTS IN FOOD

Santanu Deb-Choudhury[1*], Jessica Gathercole[1], Stephen Haines[1], Anita Grosvenor[1], Duane Harland[1], Erin Lee[1], Ancy Thomas[1], Mustafa Farouk[2], Stefan Clerens[1,3]

[1]Food & Bio-based Products, AgResearch Lincoln Research Centre, Private Bag 4749, Christchurch, New Zealand
[2]Food & Bio-based Products, AgResearch Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[3]Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand
[*]Corresponding author (phone: +64-3-321-8758; fax: +64-3-321-8811; e-mail: Santanu.deb-choudhury@agresearch.co.nz)

***Abstract*- The peptide compositions after simulated gastrointestinal digestion of powdered meat samples (pH 5.58, from 18 month old steers) and of enzymatically produced meat hydrolysates were compared using label-free quantitative analysis. Principal component analysis showed a clear distinction in the number and type of peptides produced depending on whether meat powder or hydrolysed meat was digested. Statistical analysis using ANOVA of samples taken from different time points during the simulated gastrointestinal digestion revealed significant differences ($p<0.01$) in the intensities of 409 compounds when meat hydrolysate was digested as compared to only 68 when meat powder was digested. Bioactivity profiling showed that numerous cryptides were generated during simulated digestion of the meat hydrolysate. Peptide flavour analysis revealed 70% lower flavour peptides present in the meat powder compared to the meat hydrolysate sample.**

**Key Words - Bioactivity, simulated gastrointestinal digestion, mass spectrometry.**

## I. INTRODUCTION

Protein hydrolysates are important in the food industry due to their versatility of use, from flavour enhancers to functional ingredients. Digestion may release peptides that display functionality in addition to their nutritive value, such as bioactivity. Functional peptides may also be released during food processing [1]. However, passage through the gastrointestinal tract drastically alters peptide profiles and may either enhance or limit their functional characteristics. Bioconversion of meat proteins using a combination of enzymes has been used in this study to produce an industry-relevant hydrolysate. A quantitative comparison of the peptide profiles after simulated gastrointestinal digestion of the hydrolysate *vs* the meat powder determined the influence of digestion on the peptide profiles generated. The study's objective was to evaluate changes caused by *in vitro* enzymatic processing of meat proteins, aiming ultimately to relate these changes to functionality.

## II. MATERIALS AND METHODS

*Sample preparation*

- Meat powder (MP): Bovine meat (pH 5.8) minced, freeze-dried, and milled at 4 °C.
- Meat hydrolysate (MH): MP digested (3 h at 55 °C) with Protomex (enzyme:substrate 1:125) followed by 1 h with Flavourzyme (both Novozyme) (enzyme:substrate 1:125), filtered then freeze dried.

*Simulated gastrointestinal digestion*

- Gastric digestion: Performed for 5 min using pepsin (Sigma P6887); Enzyme:substrate:1:20, pH 3.0.
- Intestinal digestion: The above gastric digest was allowed to proceed for an additional 55 min followed by 30 min pancreatin digestion (Sigma P1750); Enzyme:substrate:1:8, pH 7.0.
- The meat powder and the meat hydrolysate before the start of gastric digestion were used as controls.

*LC-MS/MS:* Samples were analysed using nanoflow LC-MS directly interfaced to a maXis HD Q-TOF (Bruker) mass spectrometer using automated information-dependent acquisition. Scheduled precursor lists featured values that differed significantly in intensity between samples. Subsequent runs with LC-MS/MS were used for peptide identification.

*Label-free quantitative analysis:* Quantitation was performed with ProfileAnalysis (Bruker). All charge states, as well as $M+NH_4$, M+K and M+Na adducts were combined for each compound. One way ANOVA analysis identified compounds that differed significantly ($p \leq 0.01$) between the control, gastric and intestinal digestion for both sample types.

*Identification:* Fragmented compounds data were imported into PEAKS Studio 8.0 [2] and searched against the SwissProt *Bos taurus* database with and without pepsin specificity.

*In silico analysis of bioactive peptides:* Custom VBA macros were used to search for putative bioactive matches of peptides from 39,900 peptide entries compiled from various databases including BIOPEP, PeptideDB, APD2 and EROP.

## III. RESULTS AND DISCUSSION

*Label-free quantitative analysis:* Statistical analysis revealed that the abundance of 409 compounds differed significantly ($p < 0.01$) when meat hydrolysate was digested, and only 68 when meat powder was digested. Those affected by meat hydrolysate digestion were predominantly found to be from the proteins actin, collagen, creatine, cytochrome c and myosin, while those from meat powder were predominantly from actin (Table 1). The increased digestion-induced variance in meat hydrolysate may indicate that the meat hydrolysate is more easily cleaved by digestive enzymes. These results thus indicate that the meat hydrolysate is a potential rich source of bioavailable peptides.

Table 1. Number of peptides significantly differing in intensities ($p \leq 0.01$) after digestion at the gastric (G) and intestinal (I) digestion phases.

| Protein | MH (G) | MH (I) | MP (G) | MP (I) |
|---|---|---|---|---|
| Actin | 85 | 68 | 12 | 16 |
| Collagen | 7 | 15 | | |
| Creatine | 14 | 7 | | |
| Cyt. C | 2 | 1 | | |
| Myosin | 82 | 30 | | 2 |

***Bioactivity analysis:*** *In silico* analysis tallied sample peptide matches to bioactive sequences in the bioactive peptides database (Table 2).

Table 2. Potential bioactivity related to physiology as determined in the meat powder and meat hydrolysate samples after simulated digestion.

| Physiological bioactives | Gastric MP vs MH | Intestinal MP vs MH |
|---|---|---|
| Antioxidative | 7 < 54 | 2 < 32 |
| Antithrombotic | 1 < 35 | 1 < 33 |
| ACE inhibit | 60 < 569 | 60 < 447 |
| DPP-IV inhibitor | 115 < 910 | 98 < 626 |
| Memory | 0 < 11 | 0 < 9 |
| Stimulating | 8 < 148 | 14 < 72 |

This gave the numbers of cryptides, i.e. bioactive peptides located within larger sample peptide sequences. Of particular interest were activities associated with inhibition of angiotensin-converting enzyme (ACE), inhibitors of dipeptidyl-aminopeptidase IV (DPP-IV), antithrombotic, antioxidative, memory regulator and stimulating. DPP-IV and ACE inhibitory sequences were predominant in peptides from both the protein sources. In the meat powder sample, the numbers of cryptides were on average 86-90% lower compared to the meat hydrolysate sample at both digestion phases.

Table 3. Tastants (number of peptides) as determined in the meat powder and meat hydrolysate samples

| Flavour | Meat | Hydrolysate |
|---|---|---|
| Bitter | 101 | 318 |
| Salty | 7 | 50 |
| Sour | 25 | 114 |
| Sweet | 66 | 163 |
| Umami | 36 | 143 |
| **Total** | **235** | **788** |

Matches to peptides with sensory properties pertaining to taste were also observed in these samples. Table 3 is representative of the various flavours detected. On average a lower percentage (70%) of these flavour peptides were present in the meat powder than in the meat hydrolysate sample.

## IV. CONCLUSION

Simulated gastrointestinal digestion of meat hydrolysate indicates it to be a potentially better substrate for bioavailable peptides with associated bioactivity than meat itself.

## ACKNOWLEDGEMENTS

We gratefully acknowledge funding from the NZ Ministry of Business, Innovation and Employment (MBIE) via the AgResearch core-funded Red Meat Combifoods Programme (Contract # ex-FRST/MSI C10X1005).

## REFERENCES

1. Hartmann, R. & Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. Current Opinion in Biotechnology 18: 163-169.
2. Ma, M., Zhang K., Hendrie, C., Liang, C., Li, M., Doherty-Kirby, A. & Lajoie, G. (2003). PEAKS: Powerful Software for Peptide De Novo Sequencing by MS/MS. Rapid Communications in Mass Spectrometry 17:2337-2342.