Advanced characterisation and evaluation of meat hydrolysates as functional ingredients in food

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Abstract- Meat hydrolysate produced by enzymatic hydrolysis of meat powder (pH 5.58, obtained from animals aged 18 months at the time of slaughter) was compared to non-hydrolysed meat powder. Amino acid analysis, focusing on nutritive qualities, indicated that the cross-linked amino acids lanthionine increased by 43%, while lysinolalanine decreased by 30% in the hydrolysate sample. Increased levels of hydroxyproline (65%) and hydroxylysine (32%) indicated a high amount of collagen in the hydrolysate. Proteomic analysis of samples from simulated gastro-intestinal digestion of the meat powder and meat hydrolysate, targeted the formation of redox and other modifications including early stage Maillard reaction products. Oxidative modification levels were similar in both sample types, showing that no unwanted oxidative protein modifications were introduced during the hydrolysis process, although more Maillard reaction products were seen in the hydrolysate sample. Further studies on potential bioactive properties were also performed. *In silico* comparison of bioactivity identified a higher percentage of bioactive peptides in the hydrolysate sample, indicating that the hydrolysate sample may be a better substrate for the early digestive release of these peptides. These results were used to identify functional properties of meat hydrolysates and compare them with those of meat powder, with the ultimate goal of using these hydrolysates as added-value ingredients in meat-based products.

Key words: Meat hydrolysate, amino acid analysis, redox modifications, bioactivity, *in silico* digestion analysis

I. INTRODUCTION

Hydrolysis of proteins into amino acids and peptides is an essential step for absorption of food proteins by enterocytes [1]. Protein bioavailability is linked to digestibility and is hence a determining factor in the nutritional quality of food [2]. Modifications in food proteins not only influence the nutritional value but also organoleptic properties such as taste, flavour and colour. The challenging task of detecting modifications in proteins and peptides from food substrates and tracking molecular changes is possible using advanced proteomic techniques, including mass spectrometry combined with tool-specific bioinformatics. The evaluation of protein modification pathways is now possible and can provide valuable information on the effects of food processing [3]. Here we demonstrate the power of this new approach using hydrolysate produced by enzymatic hydrolysis of bovine meat powder compared to the meat powder itself to evaluate prospective changes caused by processing to digestibility relevant properties. The ultimate aim of the study was to identify favourable functional properties of proteins and peptides that could eventually be used for meat based product development using these as ingredients.

II. MATERIALS AND METHODS

Sample preparation

- Meat powder: produced by mincing fresh bovine meat (pH 5.8) at 4 °C followed by freeze-drying and milling at 4 °C.
- Meat hydrolysate: produced by digesting meat powder at 55°C first with Protomex (Novozyme) at an enzyme to substrate ratio of 1:125 for 3 h followed by Flavourzyme (Novozyme) at an enzyme to substrate ratio of 1:125 for 1 h. Enzyme reaction was stopped by exposing the digest to 95°C for 12 min. The soluble fraction from the digest was filtered and then freeze dried.

Simulated digestion

Both the meat powder as well as the meat hydrolysate were used as substrates during the simulated digestion experimentation.

- Gastric digestion (60 min): performed using pepsin (Sigma P6887) at 37°C; pH 3; Enzyme to substrate ratio used was 1:20. Pepsin activity was stopped by raising the pH to 7. The digest was then further subjected to intestinal digestion.

- Intestinal digestion (30 min): performed using pancreatin (Sigma P1750); Enzyme to substrate ratio used was 1:8.

Amino acid analysis: The lyophilized meat powder and meat hydrolysate samples were subjected to amino acid analysis using HPLC after HCl vapour hydrolysis at 110 °C for 24 h followed by AccqTag derivatisation [4].

LC-MS/MS analysis: Samples were analysed using nanoflow LC-MS/MS, performed on a nano-Advance (Bruker) HPLC directly interfaced to an amaZon speed ETD (Bruker) mass spectrometer using automated information dependent acquisition.

Data analysis: Peak lists per sample were imported into ProteinScape (Bruker Daltonik) and queried against *Bos taurus* sequences in the NCBInr database (release date: 25th May 2014). A no enzyme Mascot search was performed with tolerance for up to two missed cleavages.

Statistical analysis: Data were analysed using the statistical functions of Microsoft Office Excel 2013.

In silico analysis of bioactive peptides: Custom VBA macros were used in Microsoft Excel 2013 to search for matches of peptides obtained from samples against a database containing 16,021 peptide entries compiled from the BIOPEP, PeptideDB and APD2databases.

III. RESULTS AND DISCUSSION

The potential nutritive qualities of the meat powder versus the meat hydrolysate were studied using various approaches as follows:

Amino acid analysis: The levels of almost all the amino acids increased in the hydrolysate sample compared to the meat powder sample. These are reflected in the total protein increase of 5.4% in the hydrolysate sample. These increases may be a result of the removal of non-proteinaceous material such as fats and fatty acids during the procedure used for generating the meat hydrolysate. Crosslinked amino acids such as lanthionine and lysinolalanine that may be formed during meat processing at high temperatures were also monitored [5]. Although lanthionine increased by 43%, lysinoalanine decreased by 30% in the hydrolysate sample. However, their levels were comparatively lower than other amino acids. An increased level of hydroxyproline (65%) and hydroxylysine (32%) indicated a high amount of collagen in the hydrolysate (Fig. 1).

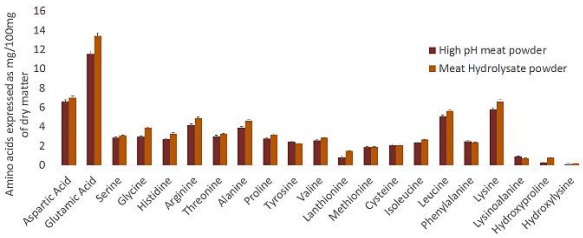

Figure 1. Amino acid profiles of high pH meat and meat hydrolysate samples. Error bars represent standard error of the means

Bioactivity assay: Prediction of the bioactivity of peptides that may be released from meat by proteolysis is very relevant to potential value-adding applications of this meat product. LC-MS/MS analyses of sequences of peptides with potential bioactive properties in the meat powder and in the meat hydrolysate were performed after simulated gastro-intestinal digestion. *In silico* analysis was used to compare the frequency of peptide sequence matches to databases containing bioactive sequences (Fig. 2). Results were compiled using partial matches, i.e. sequences of sample peptides located within sequences of peptides in the database [6]. Of particular interest were activities associated with angiotensin converting enzyme, 'ACE inhibition' that have a positive effect on the cardiovascular system; 'Inhibitors' of other enzymes such as dipeptidyl-aminopeptidase IV (associated with glucose metabolism); 'Antithrombotic', counteracting thrombosis, a pathological conditions that involves blood clots; 'Antioxidative', responsible for radical scavenging and preventing peroxidation of lipids and oxidation of proteins and DNA; 'Stimulating', associated with increased glucose uptake in muscles and also influencing release of vasoactive substances by endothelial cells linked to antiatherogenicity.

ACE inhibitory activity was predominant in peptides from both the protein sources. In the meat hydrolysate sample, as digestion progressed from gastric to intestinal, the levels of all the potentially bioactive peptides decreased. However, this trend was reversed in the meat powder sample. A reason for this could be that the meat hydrolysate sample was more easily digested by pepsin used in the simulated gastric digestion, but on additional intestinal digestion using pancreatin, these peptides were further hydrolysed, thus cleaving potentially bioactive peptide sequences. In the case of the meat powder sample, the average size of

peptides reduced after the intestinal digestion compared to the gastric digestion stage. This could have resulted in better peptide identification by the mass spectrometer with an apparent rise in bioactivity. After gastric digestion, the levels of bioactive peptides were higher in the hydrolysate sample, indicating the hydrolysate to be potentially a better substrate for the early release and bioavailability of bioactive peptides.

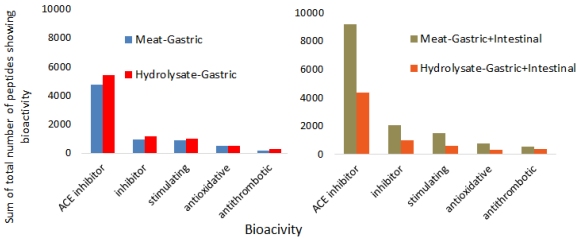

Figure 2. Potential bioactivity as determined in the meat only and meat hydrolysate samples after simulated digestion

Modification analysis: The total weighted oxidative modification scores, using previously published procedures [7], obtained from the meat hydrolysate and the meat powder samples are shown in Fig. 3. Oxidative modification levels were determined to be low in both the meat powder samples and the meat hydrolysate samples at various stages of the simulated gastro-intestinal digestion, indicating that no unwanted oxidative protein modifications were introduced during the hydrolysis process. The oxidative score of the meat powder increased as digestion progressed to the intestinal stage, possibly as modified peptides were progressively released from the substrate. Modifications other than oxidative were also monitored, e.g. deamidation, dehydration and the formation of pyroglutamic acid etc., including early-stage Maillard reaction products (MRPs). The levels of these modifications were also similar in both the sample types. The highest percentage of MRPs was detected in the meat hydrolysate after the intestinal digestion stage which accounted for twenty percent of the total amount of other modifications detected in the sample. In the meat powder sample too, after intestinal digestion, MRPs accounted for three percent of the total amount of other modifications detected in the sample. From these observations it can be concluded that although the formation of MRPs tends to increase (albeit at low levels) after the hydrolysis procedure, there are no apparent changes in other modifications.

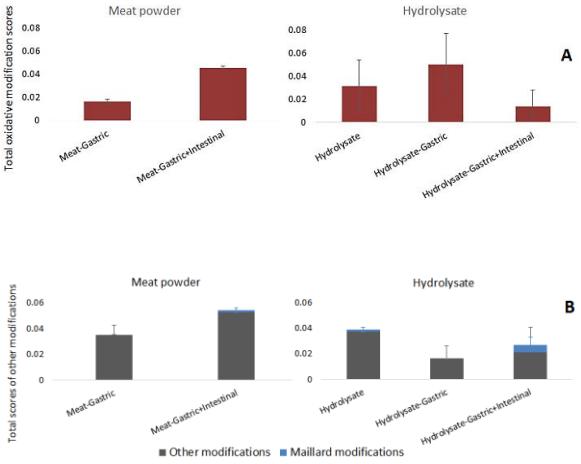

Figure 3. Comparison of scores of (A) total oxidative modifications and (B) other modifications including Maillard reaction products in meat powder and meat hydrolysate samples. Error bars represent standard error of the mean.

IV. CONCLUSION

The overall results from this study have shown that meat hydrolysate could be used as a value-added ingredient in food formulations due to the favourable functional properties associated with peptides generated through the hydrolysis procedure. No discernable change in the nutritional quality, as determined by amino acid profiling was found in the hydrolysate when compared with meat powder. Also no unwanted modifications that may reduce bioavailability of proteins/peptides in the hydrolysate were identified through redox modification studies. Simulated gastric followed by intestinal digestion on these protein sources indicated the hydrolysate to be a potentially better substrate for the early release and bioavailability of bioactive peptides during digestion compared to the meat only sample.

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