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Peptidomic comparison of dry aged *vs* a novel stepwise aged lean bull beef (#77)

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Introduction

Dry-ageing (DA) is a traditional ageing process for the tenderisation and preservation of meat. The advent of vacuum packaging and efficiency of transportation has resulted in a decline in the use of DA. However, recently there has been a resurgence of this technique due to the unique and concentrated flavours generated from the DA process which are favoured by discerning consumers. In general, both wet-ageing (WA) and DA can produce intense aged flavours and tenderise the meat [1]. During WA, meat is vacuum sealed in packages and stored in a refrigerated environment whereas during DA, the meat is aged using controlled temperature, relative humidity and air flow in a refrigerated environment but without the use of packaging. Although DA has been extensively studied over the last few decades, the unique flavours of dry-aged meat and the precursors contributing to the "dry-aged flavours" have not been well explored. The aim of this study was to compare the peptidomic profiles of meat that had undergone a novel DA (DA for 7 days followed by WA for 14 days) *vs* traditional DA (DA for 21 days) process to determine any impact on product quality.

Methods

Sample preparation: Lean bull beef striploins (pH=5.3) were DA in water permeable ageing bags (TUBLIN® 10, Denmark) for 21 d at 2 ˚C, 0.5 m/s air speed and 75% humidity and compared with stepwise aged samples (DA 7 d + WA 14 d). The unaged samples served as controls.

LC-MS/MS: Peptides were extracted from beef samples using 5% acetonitrile. The extracts were ultrafiltered using a 10 kDa cut-off filter and the peptides analysed using a nanoflow LC-MS directly interfaced to a maXis impact HD Q-TOF mass spectrometer (Bruker). Runs with MS/MS data acquired using automated data-dependent acquisition in CID mode were included for peptide identification.

Identification: Fragmented compounds data were imported into PEAKS Studio 8.5 [2] and interrogated without any enzyme specificity against the *Bos taurus* taxonomy of the UniProt database. A semi-quantitative approach using spectral counting of peptides was used to determine the differences between the two ageing treatments and compared against an unaged control sample. Two biological replicates were used per sample and a one-sample t-test was performed to establish statistical significance using R (version 3.2.2).

Results

Peptide analysis: Spectral counting for semi-quantitation of proteins was used to evaluate differences in peptide numbers derived from each protein from the aged beef samples in comparison to unaged controls. The rationale was firstly to identify differences in the peptide profiles that may have arisen due to the different conditions used for ageing the samples and to determine if the stepwise ageing procedure was comparable to the DA only. The higher spectral counts of the aged samples compared to the unaged control (Fig. 1) reflects enhanced proteolytic cleavage of meat proteins during the ageing processes. Both ageing procedures displayed very similar degrees of hydrolysis of a range of proteins, indicating comparable proteolytic activity (Fig. 1).

Taste analysis: As a proxy for sensory testing of taste properties, the bitterness index based on average hydrophobicity or *Q* value [3] of peptides from proteins with significant differences between the aged and unaged samples, was calculated. Overall only 5% of peptides from the DA only and 6.5% from the stepwise aged samples had Q values > 1400 cal/residue, indicating bitterness. Peptides from nebulin contributed most towards the bitterness index, followed by troponin T (Fig. 2).

Conclusion

The peptidomic results strongly indicate that a reduction in the DA time from 21 d to 7 d followed by a wet-ageing period for 14 d can produce comparable peptide profiles, possibly indicating similar product qualities and greater economic viability for the stepwise ageing technique.

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Notes

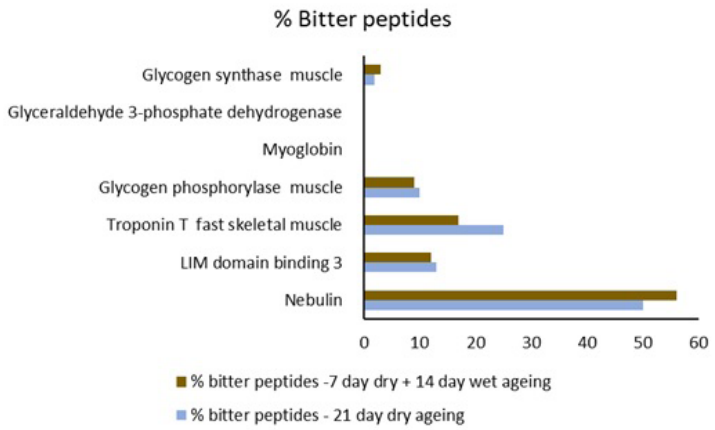

Figure 2.
Percent bitter peptides released from the proteins that differ significantly between the aged and the unaged samples

Notes

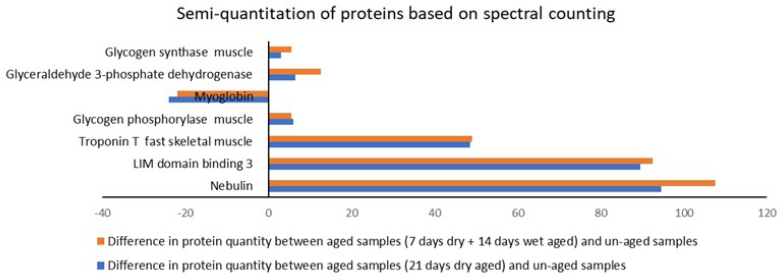

Figure 1.
Semi-quantitation of proteins, based on spectral counting, between two different aged beef samples and un-aged control samples

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