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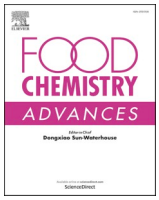

A peptidomic approach to discover the potential biomolecular signatures discriminating in-bag dry- and wet-aged lamb

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ABSTRACT

The study assessed the peptidomic profile of in-bag dry-aged (2 °C, RH 75 %, 0.5 m.s^{-1}, $n = 30$) and wet-aged (-1.5 °C, $n = 30$) lamb with high and low levels of lipid oxidation. A total of 1056 low molecular weight peptides (< 10 kDa) were identified in aged lamb samples, using LC-MS/MS and label free quantification (data are available via ProteomeXchange with identifier PXD047738). Partial Least Squares – Discriminant analysis highlighted that all discriminating peptides in dry-aged lamb were from titin, whereas discriminating peptides in the wet-aged samples were predominantly from nebulin, myotilin, collagen type IV, LIM domain binding 3, and heat shock protein family B. Comparison of peptides from aged lamb meat with high- and low-levels of lipid oxidation showed that most discriminatory peptides within an ageing method are derived from oxidatively modified peptides from creatine kinase. These peptides could thus potentially be used as signature biomolecules to discriminate ageing techniques and indicate lipid oxidation levels.

1. Introduction

Dry-ageing of fresh meat has become a value-adding strategy for the meat industry to explore alternative merchandising opportunities (Kim et al., 2018; Zhang, Yoo, Ross & Farouk, 2022). Dry-aged meat is a niche product favoured by meat purveyors for its enhanced buttery, nutty and meaty flavour compared with the widely available wet-aged equivalents (Li, Babol, Bredie, Nielsen, Tománková & Lundström, 2014; Zhang et al., 2022). As a common post-mortem tenderisation practice, wet-ageing is accomplished in moisture-impermeable vacuum bags under refrigerated conditions. While the typical processing for dry-ageing is carried out in an ageing chamber/chiller with well-controlled conditions of temperature, humidity and air velocity for 3–4 weeks (Kim et al., 2018).

A series of physical and biochemical reactions occur during dry-ageing, which includes dehydration, proteolysis, lipolysis and oxidation (Zhang et al., 2022). Several factors contribute to the formation of unique dry-aged meat flavour, such as the extent of dehydration (Lee, Choe et al., 2019; R. Zhang et al., 2023) and the generation of volatile compounds (Lee, Lee, Yoon, Kim & Jo, 2021) and metabolites such as free amino acids and peptides from the dry-ageing process (Kim, Kemp & Samuelsson, 2016; Zhang, Ross, Yoo & Farouk, 2021a). As meat ages, significant changes in the composition of flavour precursors are observed. Dry-aged flavour results from the release of free amino acids, peptides, and the breakdown of ribonucleotides, to yield inosine 5′-monophosphate, guanosine monophosphate, inosine, and hypoxanthine (Lee, Choe et al., 2019; Zhang, Yoo & Farouk, 2021b). Many of these changes are due to the activity of various hydrolases including the Ca^{2+}-dependent calpain proteases and the cathepsins implicated in the production of flavour peptides (Nishimura, 1998). Several amino acids, including tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine and leucine, have been reported to be more abundant in dry-aged than wet-aged beef (Kim et al., 2016). These breakdown products further contribute to the intense meaty, nutty and roasted flavour of dry-aged beef when cooked (Li et al., 2014; Zhang et al., 2022).

Dry-ageing is an expensive process due to the larger space and

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greater energy processing requirements and the significant weight losses from dehydration (10–15 %) and trimming of the "crust" formed on the meat surface; resulting in higher production costs and therefore higher product prices compared to the wet-aged equivalents. Most consumers are not familiar with the quality and characteristics of dry-aged meat, therefore it is difficult for consumers to identify and discriminate dry-aged meat from the wet-aged, especially when different dry-ageing regimes have been applied and the well-trimmed meat is packaged using vacuum or skin packaging for final commercialisation. Application of advanced analytical techniques may identify the biomolecular signature for discriminating dry- and wet-aged meat facilitating appropriate labelling of high value dry-aged meat.

Previous studies have reported the use of peptides as markers for monitoring the meat ageing process (Fu, Young & Therkildsen, 2017; R. Zhang et al., 2023; Zhang et al., 2021a, 2021b). Peptides have higher stability to thermal treatments than intact proteins, which potentially allow for the identification of ageing-specific markers and for further understanding of the underlying biochemical changes during meat ageing. Peptidomic approaches have been suggested as a useful tool for authenticating sheep meat from other animal species (R. Zhang et al., 2023). Furthermore, detection of marker peptides produced from enzymatic hydrolysis using label free relative quantification through liquid chromatography coupled to mass spectrometry (LC-MS) has been successfully applied for the determination of species-specific peptides from meat, with high sensitivity and efficiency (Montowska & Fornal, 2017). Interestingly, recent studies have also demonstrated the potential of using small peptides in meat as biomolecular signatures for dry-ageing (Choe, Park, Lee & Jo, 2020; R. Zhang et al., 2023; Zhang, Ross, Yoo & Farouk, 2021a, 2021b).

Therefore, the aim of the present study was to investigate the feasibility of utilising a peptidomic approach, specifically through label free quantification of the low molecular weight peptides (< 10 kDa), to identify the potential biomolecular signatures discriminating in-bag dry- and wet-aged lamb. In addition, the peptide profiles of lamb with different levels of lipid oxidation from both ageing methods, were also evaluated to improve understanding of the ageing process to facilitate monitoring procedures for quality improvement.

2. Materials and methods

2.1. Meat samples and ageing process

This study was carried out complementary to Zhang, Yoo, Realini, Staincliffe and Farouk (2021) and the similarities in the experimental design and the sample sets are acknowledged. Lamb hindlegs (shank and chump off, $n = 60$) were collected from thirty lamb carcasses (Ram, approx. 46 weeks old) on the day of slaughter. Paired legs (left and right) from the same animal were randomly assigned to two ageing treatments: in-bag dry-ageing ($n = 30$) or wet-ageing ($n = 30$). Briefly, in-bag dry-ageing was carried out in water permeable ageing bags (TUBLIN® 10, 50 μm thick, polyamide mix with water vapor transmission rate 920 g/50 μ/m^2/24 h at 7 °C, 50 % RH, and oxygen transmission rate 660 g/m^2/24 h at 7 °C, 50 % RH, TUB-EX ApS, Denmark) at 2 ± 0.5 °C, 0.5 m.s^{-1} air velocity and relative humidity of 75 ± 5 %. Wet-ageing of lamb legs using water impermeable barrier bags (Cryovac® A600 barrier bag, oxygen transmission rate 20–50 g/m^2/24 h at 23 °C, Sealed Air®, New Zealand) was performed at −1.5 ± 0.5 °C. After 21 days of ageing time, lamb chops (1.5 cm thickness, consisting of four main muscles: *m. semimembranosus, m. biceps femoris, m. vastus lateralis*, and *m. rectus femoris*) were taken from the same anatomical position of the lamb legs (both wet- or dry-aged) with no further trimming. The deboned lamb chops were then freeze-dried (~3 days) and ground into fine powder for further peptidomic analysis.

Degree of lipid oxidation was determined as in our previous study (Zhang et al., 2021) using thiobarbituric acid reactive substances (TBARS) assay (Buege & Aust, 1978). Paired lamb legs were further divided into two groups with either high (> 1.0, 17 carcasses, $n = 34$) or low (< 1.0, 13 carcasses, $n = 26$) TBARS values as measured in both wet and dry-aged samples to investigate any potential relationships between the degree of lipid oxidation and the production of peptides.

2.2. Extraction and quantification of peptides

Peptide extracts ($n = 60$) were obtained from lyophilised and pulverised meat powder from both ageing treatments. Approx. 20 mg of meat powder was mixed with appropriate volume of MilliQ water on a vortex mixer for 60 s then homogenised in a sonicator for 5 min to obtain a sample mixture with the final concentration of 20 mg.mL^{-1}. The homogenate was then spun at 1000 rpm for 10 min. The liquid fraction (top layer) was obtained and mixed with 5 % acetonitrile (1:3, v/v) before centrifuged at 15,000 rpm for 10 min. An aliquot of clear supernatant containing water soluble fraction of lamb samples was transferred in to a Nanosep® 10 K Omega™ tube (Pall Corporation, USA) and spun at 5000 g for 90 min at 4 °C. The peptide concentration of the ultrafiltrate was determined using a peptide assay kit (Pierce™ quantitative colorimetric peptide assay, Thermo Fisher Scientific, San Jose, CA, USA). The peptide concentration was calculated from an average of triplicate measurements. An aliquot of the peptide solution containing 38 μg peptides was taken and spun in a vacuum evaporator at 40 °C to remove liquid fraction. The peptide sample was re-suspended in 50 μL formic acid (0.1 %) for label free quantification.

2.3. Peptide profiling

2.3.1. Mass spectrometry

LC-MS was performed on a nanoflow Ultimate 3000 UPLC (Dionex) coupled to an Impact HD mass spectrometer equipped with a CaptiveSpray source (Bruker Daltonik, Bremen, Germany). For each sample, 1 μL of the sample was loaded on a C18 PepMap100 nano-Trap column (300 μm ID x 5 mm, 5 μm 100 Å) at a flow rate of 3000 nL.min^{-1}. The trap column was then switched in line with the analytical column ProntoSIL C18AQ (100 μm ID x 150 mm 3 μm 200 Å). The reverse phase elution gradient was from 2 % to 20 % to 45 % B over 60 min, total 84 min at a flow rate of 1000 nL.min^{-1}. Solvent A was LCMS-grade water with 0.1 % Formic acid; solvent B was LCMS-grade Acetonitrile with 0.1 % Formic acid.

The LC was directly interfaced with a captive spray ion source (3.0 L.min^{-1} dry gas, operated at 1500 V) to a high-resolution Impact HD quadrupole-time-of-flight (Q-TOF) (Bruker Daltonics) mass spectrometer. To profile protein expression patterns, the analytes were detected via MS-only mode in positive ion mode, with a mass range between 130 – 2200 *m/z* and a sampling rate of 2 Hz. To link the expression levels with identifications, a pool of per treatment was created, and these pooled samples were run via LC-MS/MS with data-dependent auto-MS/MS mode with the following settings: the same LC parameters as described before, a full scan spectrum, with a mass range of 150 – 2200 *m/z*, was followed by a maximum of ten collision-induced dissociation (CID) tandem mass spectra at a sampling rate of 2 Hz for MS scans and 1 to 20 Hz for MS/MS. Precursors with charges 1+ to 5+ were preferred for further fragmentation and a dynamic exclusion of 60 s was set.

Following the LC-MS run, the Q-TOF data were further analysed with Compass DataAnalysis 4.4 software (Bruker Daltonics) to evaluate the LC chromatogram and the overall quality of both MS1 and MS2 spectra.

2.3.2. Peptide identification

The PEAKS *X*+ Studio data analysis software package (Bio informatic Solutions Inc, Waterloo, Canada) was used to analyse the LC-MS/MS data. The raw data were refined by a built-in algorithm which allows association of chimeric spectra. The peptides were identified with the following parameters: a precursor mass error tolerance of 10 ppm and fragment mass error tolerance of 0.05 Da were allowed, the Uniprot_Ovis aries database (v2018.07, 27,885 sequences) was used, the

minimum peptide length was set to 3 amino acids long and an unspecific digestion was specified, as no enzyme was added to generate the peptides. Oxidation (M), phosphorylation (STY) and deamidation (NQ) were chosen as variable modifications. A maximum of 3 post-translational modifications (PTMs) per peptide was permitted. False discovery rate (FDR) estimation was made based on decoy-fusion. An FDR of < 5 % on peptide spectrum match level and a PTM A-score of 100 was considered adequate for confident peptide identification.

2.3.3. Label free quantification

To quantify the peptide expression levels, label-free quantification (LFQ) was performed using the quantitation node of Peaks Studio *X*+ software. Here, peptide expression levels between all samples were compared. The following parameters were included: a mass tolerance error of 15 ppm and a retention time shift tolerance of 2 min was allowed. To determine the relative peptide abundance in the RT aligned samples, peptide feature based quantification was performed. Relative comparison between samples was based on the area under the curve at the MS1 level after TIC normalisation.

2.4. Statistical analysis

Peptide information from lamb chops ($n = 60$) were obtained from thirty animals, applying two ageing techniques (in-bag dry- and wet-ageing) and selecting two levels of lipid oxidation (low and high). We followed a multivariate data analysis approach (R version 4.2.1, R Core Team 2021) to identify peptides driving the discrimination between the lamb samples in a supervised PLS-DA (Partial Least Squares – Discriminant Analysis) from MixOmics (Rohart et al., 2017) (Fig. 1). We then proceeded to identify peptides whose abundance differed depending on the in-bag dry- or wet-ageing process by fitting a negative binomial model to the sample groups and calculating the significance of the difference after correcting for multiple testing using Benjamini-Hochberg. Finally, peptides with a log fold change higher than 2, and a mean intensity above 10,000 cts were listed.

3. Results

This study evaluated the feasibility of utilising a peptidomic approach to discover potential biomolecular signatures that distinguish between in-bag dry-aged and wet-aged lamb. Furthermore, the peptide profiles of lamb with different levels of lipid oxidation from both aging methods were also evaluated to improve understanding of the ageing process and facilitate quality improvement monitoring procedures. The present study followed a dual approach as outlined in Sections 3.1 and 3.2.

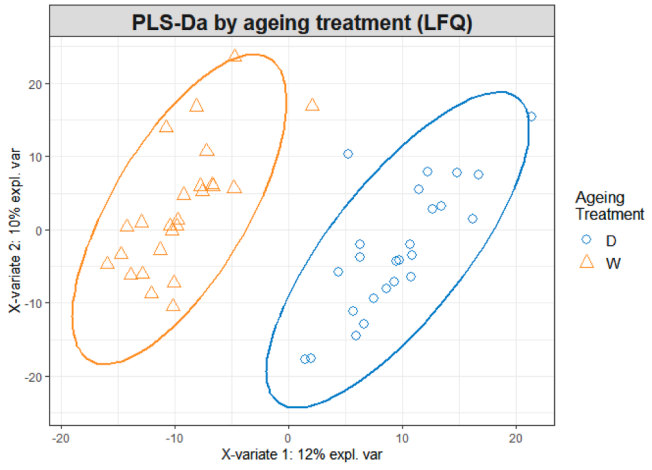

Fig. 1. PLS-DA score plot of in-bag dry- (Blue circle) and wet-aged (Orange triangle) lamb samples.

3.1. Differences in peptide profiles

To investigate differences in peptide profiles between all 60 samples, a multivariate data analysis approach was performed using PLS-DA. As shown in Fig. 1, the two ageing techniques can be successfully discriminated based on the abundance levels of all 1056 quantified peptides in lamb samples (Supplementary Table S1). The top twenty peptides that were driving the discrimination between the wet- and dry-aged groups are listed in Fig. 2.

Next, the lipid oxidation status of the samples was also considered. When a comparison between peptide profiles of these four treatment groups (ageing methods × lipid oxidation levels) is performed, clustering is still mostly driven by the in-bag dry- *vs.* wet-ageing status, although a visual distinction between low and high lipid oxidation levels was evident on the second principal component (Fig. 3). The top twenty peptides that were driving the discrimination of low and high lipid oxidation levels (Figure S1) regardless of the ageing process are shown in Fig. 4.

3.2. Difference in peptide abundance

A univariate analysis was performed to detect peptides whose abundance differed depending on the in-bag dry- or wet-ageing process. Two different parameters in this statistical analysis are of major importance: (i) the *P*-value: how significantly different are the peptide abundancies between the experimental groups, and (ii) the fold change: how large are the differences in peptide abundancies between the two treatment groups. The peptides of interest (Table 1) are grouped in different categories. Firstly, the peptides with a *P*-value < 0.05 and a log2 fold change greater than 2 are listed. We then proceeded to identify peptides with a fold change value greater than 2 that did not pass the significance threshold cut-off of 0.05. These peptides were further filtered to only include peptides with mean intensities above 10,000 cts (Table 1).

4. Discussion

In-bag dry- and wet-aged lamb meat were compared to determine whether these two aging processes resulted in differences in their respective peptide profiles. All discriminating peptides in the dry-aged samples were derived from the titin while the discriminating peptides from the wet-aged samples were from a group of proteins that included nebulin, myotilin (protein accession W5Q1Q5), collagen type IV (W5QCP9), LIM domain binding 3 (W5NR35) and heat shock protein family B (W5P4P2).

It is widely agreed that the development of tenderness during ageing primarily arises from post-mortem proteolysis of key structural proteins (Lametsch, Karlsson, Rosenvold, Andersen, Roepstorff & Bendixen, 2003) and the disruption of the sarcomere structure, due to Z-disks degradation within a few days of ageing (Morzel et al., 2004). Titin degradation has been associated with physiological changes in post-mortem muscles. During the meat ageing process, tenderness improvement occurs in the first 7 to 14 days but continues after that albeit at a slower rate with the continuing proteolysis of Z-lines, troponin-T, titin, nebulin and desmin Huff-Lonergan Parrish Jr and Robson, (1995); Miller, (2002). Titin, having a molecular weight of over 3000 kDa, is the largest protein found in muscle extending from the Z-line to the M-line within sarcomeres. The significant increase in the peptides derived from titin identified in the in-bag dry-aged lamb in this study, indicates that a higher rate of degradation of this protein was involved due to the processing conditions of dry-ageing.

In the wet-aged samples, twice the fold change of peptides from nebulin was observed. Previously it has been shown that a high rate of nebulin degradation is aided by calpains which are Ca^{2+}-dependent

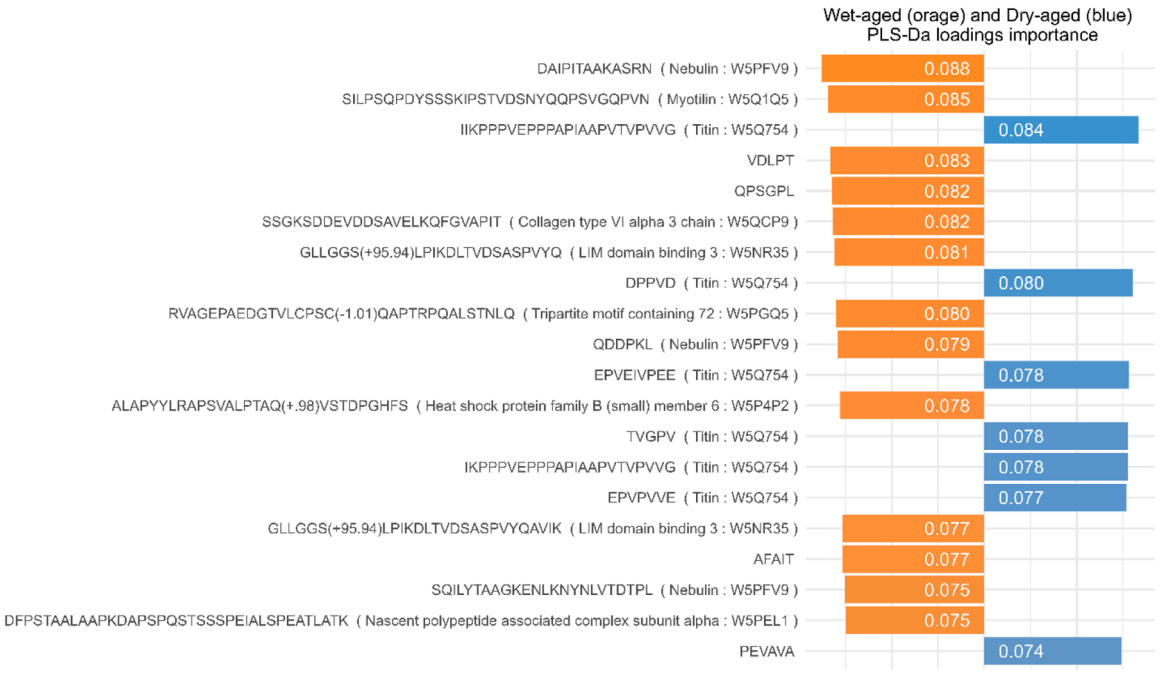

Fig. 2. Top twenty peptides (with their protein accession numbers) that are driving the discrimination between dry-aged (blue, right side) and wet-aged (orange, left side) groups based on supervised discrimination analysis (PLS-DA).

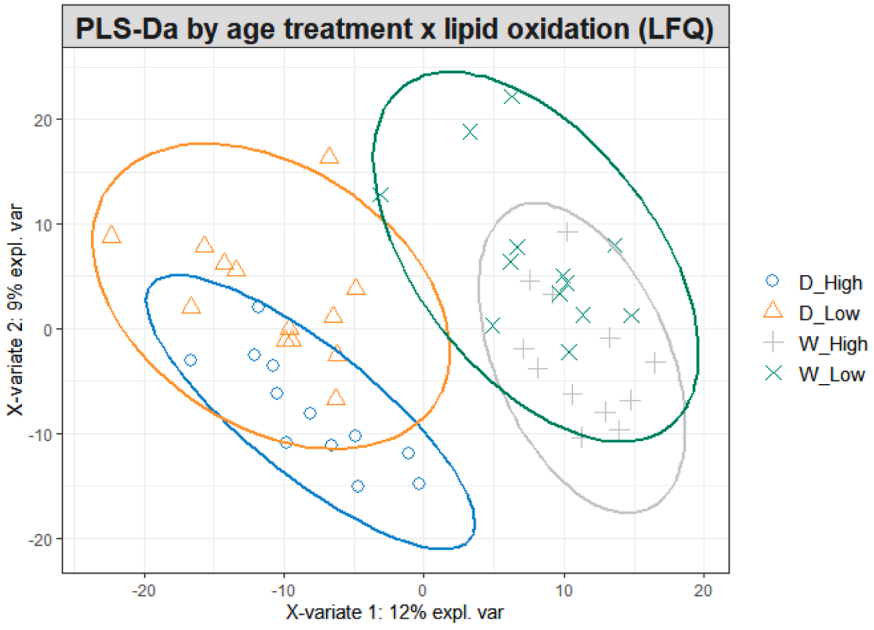

Fig. 3. PLS-DA score plot of aged lamb samples by ageing treatment and lipid oxidation. Blue circles (o): in-bag dry-aged with high lipid oxidation; Orange triangles (Δ): in-bag dry-aged with low lipid oxidation; Grey multiplication signs (×): wet-aged with high lipid oxidation; Green plus signs (+): wet-aged with low lipid oxidation.

proteases endogenous to skeletal muscle cells (Bhat, Morton, Mason & Bekhit, 2018). The degradation of nebulin leads to the disruption of the myofibrillar structure resulting in meat tenderisation (Huff-Lonergan, Zhang & Lonergan, 2010). Peptides related to myotilin (W5Q1Q5), collagen type IV (W5QCP9), LIM domain binding 3 (W5NR35) and heat shock protein family B (W5P4P2) were also significantly higher in the wet-aged lamb samples. The changes in several of these proteins can be associated with meat tenderisation during the ageing process. For example, heat shock proteins family B, belonging to small heat shock proteins (sHSP), are chaperone proteins that are abundantly expressed in muscles after slaughter (Pulford, Vazquez, Frost, Fraser-Smith, Dobbie & Rosenvold, 2008). The sHSPs are known to regulate actin polymerisation and actin-myosin interactions (Mounier & Arrigo, 2002). Thus, the expression of sHSPs prevent the aggregation of myofibrillar proteins and contribute towards the proteolytic degradation and meat tenderisation during ageing. The increased abundance of the peptide derived from sHSP in wet-aged lamb could potentially indicate a greater rate of sHSP degradation during wet-ageing, consequently diminishing its chaperone function. These changes, in turn, promote the proteolytic breakdown of actin and myosin, ultimately resulting in tender meat

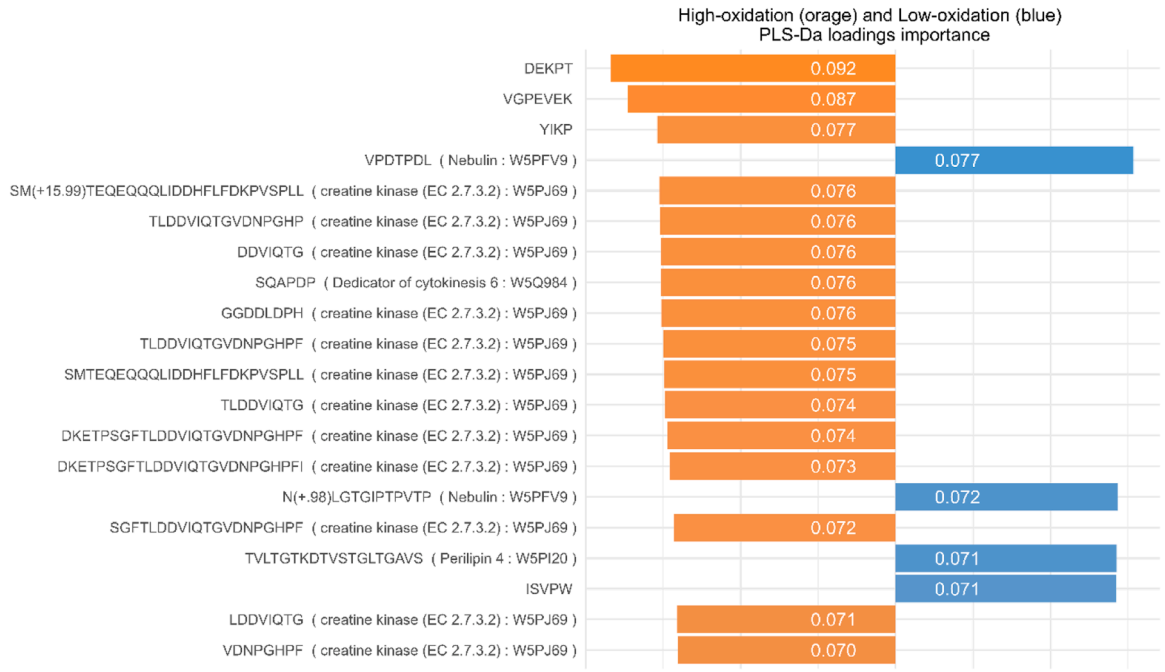

Fig. 4. Top twenty peptides (with their protein accession numbers) that are driving the discrimination of low (blue, right side) and high (orange, left side) lipid oxidation status in the dry- and wet-aged lamb meat based on supervised discrimination analysis (PLS-DA).

Table 1
Peptides identified as being significantly different based on *P*-values ($P < 0.05$) and log2fold change value > 2; and peptides of interest with a log2fold change value > 2\ and mean intensity value > 10,000 cts.

Peptide	*P*-value	log2fold	Accession	Protein name
Based on P-value (<0.05) and log2fold change > 2				
VYNV	0.0002	−7.98103	W5P9A0	Succinate-semialdehyde dehydrogenase
KFDLI	0.0014	−7.65077	W5NX73	Chaperone ATP11
Based on log2fold change >2 and intensities >10,000 cts				
ETPKYQ	1	−2.11566	W5PFV9	Nebulin
VPEPPPK	1	2.217086	W5Q754	Titin
TVGPV	1	2.305536	W5Q754	Titin
VPSR(+14.02) EPDAAQTAGLRT	1	2.462892	W5Q5Y7	Synaptopodin 2

during the wet-ageing process (Zhang, Yoo & Farouk, 2021; Li et al., 2014).

Myotilin and LIM domain binding proteins are Z-line proteins and their disruption during the ageing process could initiate physicochemical and structural changes resulting in the tenderisation of the muscle (Huff-Lonergan et al., 2010). LIM domain binding 3 protein (LDB3) peptides present in higher abundances in this study, indicate that this protein may be subjected to a different enzymatic proteolysis pathway occurring from the wet-ageing process. It has been reported earlier that post-mortem ageing over 72 h caused proteolytic cleavage of constitutive proteins of the Z-line, resulting in the weakening of the Z-line (Morzel, Chambon, Hamelin, Sante-Lhoutellier, Sayd & Monin, 2004). It has also been established that LDB3 protein sequences are mostly acted upon by aminopeptidases, carboxypeptidases and cathepsins during the curing process (Gallego, Mora, Fraser, Aristoy & Toldrá, 2014).

Almost all peptides obtained from samples of lamb with high lipid oxidation rates and regardless of the ageing process, were from creatine kinase (W5PJ69). Creatine kinase is one of the most abundant sarcoplasmic proteins in skeletal muscles but is also a substrate for proteases involved in the enzymatic proteolysis during ageing (Lametsch, Roepstorff & Bendixen, 2002) and curing/fermentation processes (López, Bru, Vignolo & Fadda, 2015). Sarcoplasmic proteins have been suggested to be more susceptible to proteolysis compared to myofibrillar fractions (López et al., 2015). Creatine kinase is mainly degraded by endogenous muscle proteases including μ-calpain during the early post-mortem period (1–2 weeks) (Purintrapiban, Wang & Forsberg, 2001) and cathepsins B and L during the extended storage and processing period (Mora, Sentandreu, Fraser, Toldra & Bramley, 2009). Peptides resulting from the proteolysis of creatine kinase may be employed as useful indicator for lipid oxidation level of aged meat. Creatine kinase proteolysis in lamb samples with high lipid oxidation levels could have also been due to higher muscle protein oxidation due to the presence of lipid peroxides. This hypothesis could be supported by the higher level of peptides derived from synaptopodin 2 (Table 1) in dry-aged lamb which was an indication of oxidative stress (Longo, Lana, Bottero & Zolla, 2015). Similarly, previous studies have shown that proteolysis of creatine kinase by cathepsin B and L in rainbow trout is minimised under non-oxidative conditions (Godiksen, Morzel, Hyldig & Jessen, 2009).

The role of endogenous proteases in proteolysis was suggested to be similar between the two ageing techniques (Zhang et al., 2022). However, protein metabolism can vary during the different ageing processes, leading to the alteration of peptide profiles, and consequently affecting the texture properties of meat. This is further supported by our previous observation on the same sample set that in-bag dry-aged lamb had slightly harder and chewier texture compared to the wet-aged equivalents (Zhang, Yoo, Realini et al., 2021). Different peptide levels resulting from the proteolysis of titin (W5Q754), LDB3 (W5NR35), myotilin (W5Q1Q5), nebulin (W5PFV9) and sHSP (W5P4P2) between the two ageing techniques could be associated with two factors: (1) the higher ageing temperature for the dry-ageing compared to the wet-aged process; and (2) the differential activities by microbial enzymes. The slightly higher ageing temperature (2 *vs.* −1.5 °C) used for dry-ageing compared to wet-ageing in this study could have contributed towards an improved proteolytic activity of muscle proteases, resulting in an increased level of small peptides derived from titin in dry-aged samples. A higher rate of proteolysis was suggested by Kim et al. (2016) for dry-aged beef at 3 °C compared to wet-aged at 1 °C due to the increased

level of free amino acids in the dry-aged beef. However, no significant difference in the shear force and sensory tenderness between the two ageing temperatures was observed in their study. Therefore, the actual impact of the slight increase in ageing temperature on the proteolytic activity in meat needs further investigation. Such an increase of amino acid concentration could be associated with the concentrating effect of moisture evaporation rather than ageing temperature during dry-ageing (Lee, Choe et al., 2019; Zhang et al., 2022). Another contributing factor could be the action of endopeptidases and exopeptidases by microorganisms (Mora, Sentandreu & Toldrá, 2010), especially those originating from mould and yeast (Choe et al., 2020; Zhang, Ross et al., 2021a). Proliferation of mould and yeast has been observed on the crust of dry-aged beef (Lee, Yoon et al., 2019; Oh, Lee, Lee, Jo & Yoon, 2019) and lamb (Zhang, Yoo, Realini et al., 2021), which could result in a unique profile of small peptide in dry-aged meat (Choe et al., 2020; Zhang, Ross et al., 2021b). The in-bag dry-ageing regime applied in this study has been shown to result in a significant increase of yeast with no impact on mould (Zhang, Yoo, Realini et al., 2021). Exogenous peptidases from yeast (*Debaryomyces hansenii*) have been previously shown to generate a range of small peptides and free amino acids during the dry-ageing process (Lee, Yoon et al., 2019). Future studies should be carried out to confirm the linkage between microbial activities and the production of specific peptides observed in this study.

5. Conclusion

For the first time, peptidomic profiling of small peptides (< 10 kDa) was performed on in-bag dry-aged lamb as compared to the wet-aged equivalents. A total of 1056 peptides were identified in both aged lamb samples. The profile of peptides during in-bag dry-ageing of lamb differed from the equivalent muscles from wet-ageing, which may be associated with different ageing conditions and activities of microorganisms. Peptides released from titin, nebulin, myotilin, collagen type IV, LIM domain binding 3, and heat shock protein family B could be the potential signature molecules for discriminating lamb from the two ageing techniques. Further, the increased levels of peptides derived from creatine kinase can be a potential indicator for lipid oxidation level of aged meat. Future studies should focus on understanding the underlying mechanisms for the observed differences in peptide profiles between ageing techniques and validating the potential of using these peptides as biomolecular signatures for in-bag dry-aged lamb.

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CRediT authorship contribution statement

Renyu Zhang: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Evelyne Maes:** Writing – review & editing, Validation, Software, Methodology, Formal analysis. **Charles Hefer:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Alasdair Noble:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Ancy Thomas:** Writing – review & editing, Resources, Methodology. **Michelle J.Y. Yoo:** Writing – review & editing, Conceptualization. **Mustafa M. Farouk:** Writing – review & editing, Methodology, Conceptualization. **Carolina E. Realini:** Writing – review & editing, Funding acquisition. **Santanu Deb-Choudhury:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) partner repository with the dataset identifier PXD047738.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2024.100664.

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