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Use of Rapid Evaporative Ionisation Mass Spectrometry fingerprinting to determine the metabolic changes to dry-aged lean beef due to different ageing regimes

Renyu Zhang[1,2*], Alastair B. Ross[3], Michelle J.Y. Yoo[2], Mustafa M. Farouk[1]

[1]Meat Quality Team, Food & Bio-based Products, AgResearch Ltd, Ruakura Research Centre, Hamilton, New Zealand.

[2]School of Science, Faculty of Health and Environment Sciences, Auckland University of Technology, Auckland, New Zealand.

[3]Proteins and Metabolites Team, Food & Bio-based Products, AgResearch Ltd, Lincoln Research Centre, Christchurch, New Zealand.

***Corresponding Author: Renyu Zhang. Email: renyu.zhang@agresearch.co.nz**

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Abstract

Rapid Evaporative Ionisation Mass Spectrometry (REIMS) was used to determine the impact of in-bag ageing regimes (stepwise-ageing at different air velocities and straight-dry-ageing) and trimming on the metabolic profile of dry-aged lean beef. Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) models based on 1705 tentatively identified m/z features were found for ageing methods (Q^2 = 0.85), ageing time (0 *vs.* 21 days, Q^2 = 0.95) and sampling locations (surface meat *vs.* trimmings, Q^2 = 0.94). No significant (P > 0.05) difference in metabolites due to air velocities. Small metabolites such as dipeptides and amino acids were more abundant, especially on the surface of untrimmed lean beef, following 21 days of straight-dry-ageing. Stepwise-ageing produced different metabolic profiles from straight-dry-ageing, suggesting that the two methods may differ in dry-aged meat quality and flavour. This work demonstrates REIMS's potential for real time differentiation of meat on processing parameters.

Keywords: REIMS; in-bag dry-ageing; stepwise-ageing; lean beef; metabolic fingerprinting.

1 Introduction

Dry-ageing is a traditional post-mortem processing technique for the preservation, tenderisation and flavour development in meat. Dry-aged beef, which would normally require an ageing period of 3-4 weeks, are commonly marketed as artisan meat with a higher price tag and intense flavour profile compared to wet-aged beef (Iida et al., 2016; Kim et al., 2018). Strong interest in dry-aged meat from meat industries and consumers has led to a need to better understand what occurs during dry-ageing and how this contributes to a value-premium meat product (Laster et al., 2008). Previous studies have demonstrated the improvement of sensory quality using dry-ageing technique, including the generation of distinct and intense buttery, nutty and meaty flavours (Li et al, 2014; Savell, 2008). However, the impact of different ageing conditions (air velocity, temperature and humidity) on the quality of dry-aged products is not well understood. Limited studies have reported variations in the quality and microbial compositions of dry-aged beef owing to air velocity (Lee et al., 2019), temperature and relatively humidity (Kim, Kemp, & Samuelsson, 2016). It is important to be able to easily determine these variations for the purpose of quality control and preventing fraud where dry-aged meat is concerned compared to the wet-aged.

The biochemical and biophysical signatures which define and discriminate dry-ageing process from wet-ageing are yet to be fully determined. Through metabolic profiling, it could be possible to identify compounds that are formed solely from dry-ageing processes, with specifications related to when these compounds appear in relation to the ageing period. Such information would guide both consumers and manufacturers over what to expect for dry-aged beef and provide a reference point for regulation, process improvement and authenticity. The potential signature could include the level of weight loss, certain volatile compounds (O'Quinn et al., 2016) and/or certain groups of metabolites such as unique peptides and amino acids (Kim et al., 2016). Several amino acids, such as tryptophan, phenylalanine,

valine, tyrosine, glutamic acid, isoleucine and leucine have been reported to be more abundant in dry-aged beef than the wet-aged presumably due to a greater extent of proteolysis during dry-aging (Kim et al., 2016).

Recently, the use of biomarker/signature-driven food testing to verify food authenticity and to assure quality, has gained traction. Several analytical platforms have been used for metabolic characterisation of meat products, mainly chromatography-mass spectrometry (LC-MS and GC-MS) (Trivedi et al., 2016) and isotope ratio mass spectrometry (IRMS) (Piasentier, Valusso, Camin, & Versini, 2003), Fourier transform infrared (FT-IR) spectroscopy (Ellis, Broadhurst, Kell, Rowland, & Goodacre 2002), and nuclear magnetic resonance (NMR) (Kim et al., 2016). However, the major disadvantages of these techniques are that they often require extensive sample preparation time and labour and require >10 minutes for enough data to be acquired, limiting the applicability of the methods outside of the laboratory environment. A high per-sample cost also inhibits the use of these methods for product development and quality control. Further, the measurements undertaken must be able to account for the complexity of meat composition due to the factors including species, breed, age, sex and type of muscle, which will require a panel of biomarker compounds rather than a single marker to adequately characterise the meat products.

One potential tool for performing rapid meat composition measurements is the Rapid Evaporative Ionization Mass Spectrometry (REIMS). REIMS has been used earlier in meat research for proof of principle work including identifying meat from different species and breeds (Balog et al., 2016), authentication of minced beef (Black et al., 2019) and the detection of boar taint in an abattoir setting (Verplanken et al., 2017). The underlying principle of REIMS is the thermal ablation of tissue samples by electric currents using a surgical knife (also called iKnife) to generate an aerosol, which is ionised allowing the ablated compounds to be detected by mass spectrometry (Ross et al., 2020). A real-time (2-3

s) determination is possible with no sample preparation needed and results in a reproducible metabolic fingerprint consisting of > 1000 detected features.

Stepwise ageing is a processing regime proposed for industrial application in order to reduce the costs associated with the traditional dry-ageing process without compromising the quality of the dry-aged products (Campbell, Hunt, Levis, & Chambers, 2001; Kim et al., 2018). Our previous study assessed the quality and sensory acceptability of in-bag dry-aged lean beef produced using stepwise ageing technique (7 days in-bag dry-ageing + 14 days wet-ageing), and compared it to straight-dry-ageing for 21 days and found that the quality and oxidative stability measured were similar (Zhang, Yoo, & Farouk, 2019, 2020). Thus, the aim of this study was to explore the potential of REIMS to determine the biomolecular difference between in-bag dry-aged lean beef produced using a stepwise-ageing process with different ageing chamber air velocities, compared with equivalent straight-dry-aged lean beef.

2 Materials and methods

2.1 Ageing regimes

This study was carried out as an extension of work previously published Zhang et al. (2019), with the same experimental design and sample set. Thirty striploins (*Longissimus lumborum*, 1-2% crude intramuscular fat, Holstein-Friesian, ~2 years old) from fifteen bull beef carcasses were collected on the day of slaughter from an abattoir and held at 12 °C until rigor. The striploins all reached normal ultimate pH range (5.32-5.36) as previously reported (Zhang et al., 2019). The 30 striploins were assigned to four ageing regimes with a control dry-ageing (T1, n = 6) and three stepwise ageing regimes (T2-T4, n = 8 for each treatment). The paired loins were assigned to different treatments with 6 single loins of different carcasses for T1 (n = 6); the remaining 24 loins of 15 carcasses (6 single loins + 18 paired loins) were then randomly allocated to treatments T2-T4 with each treatment allocated 8 loins

from different carcasses (n = 8). The Control (T1) was a straight-dry-ageing of beef using water-permeable ageing bag (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%, for 21 days; The Stepwise-ageing at three air velocities (T2) - (T4) involved: in bag dry-ageing for 7 days using the same conditions as T1, followed by wet-ageing in vacuum barrier bags (Cryovac® A600 barrier bag, Sealed Air®, New Zealand) for 14 days using air velocity of 0.5 $m.s^{-1}$ (T2); 1.5 $m.s^{-1}$ (T3) and 2.5 $m.s^{-1}$ (T4). Steak samples (2 cm thickness) were taken from unaged (day 0, T1-T4), day 7 (T2-T4) and day 21 (T1-T4) of ageing. Trimming of the dry surface (approximately 5 mm) was only performed on the steak samples on day 7 or 21 of ageing. To determine the effect of sampling locations on the metabolic profile of lean beef, another five striploins (n = 5) were assigned to T1 ageing treatment but without trimming of dehydrated surface following 21 days of straight-dry-ageing. All samples were stored at -80 °C until analysis.

2.3 REIMS

REIMS measurements were carried out using an electronic monopolar surgical knife coupled to a Waters Xevo® G2 qToF mass spectrometer with REIMS interface (Waters Corp. Wilmslow, UK). The surgical knife was set to 'cut' mode and 15 W power (Electrosurgical pencil and Erbe VIO 50C generator, Erbe Medical UK Ltd, UK). Cuts approximately 2 mm deep and 15 mm long, and 3 seconds per cut were made directly in thawed meat samples. Five technical replicates for each meat sample were acquired, with a 10 second delay between cut to allow signal to return to baseline and for the electronic knife to be cleaned by wiping with a clean tissue wetted with deionised water and scraping to avoid loss of conductivity. To determine the effect of trimming on metabolic profile, three different sampling locations were used from untrimmed steaks (n = 5): (1) surface meat/crust, but not

on the silver skin and fat, (2) trimmings: the area where post-ageing trimming is usually carried out (within 1 cm away from the surface) and (3) the centre of the steak. Samplings for the other treatments (T1-T4) were carried out at the centre of the steak. The use of five technical replicates provides both better reproducibility and spatial coverage of the meat sample. The resulting aerosol/smoke was evacuated from the sample through a transfer line into the mass spectrometer for ionisation and subsequent analysis. The mass spectral data were acquired in untargeted mode with negative ionisation between mass range of m/z 50-1500 with a scan rate of 0.5 seconds per scan. The instrument was calibrated prior to the sampling using sodium formate and run in sensitivity mode with a mass resolution of 40,000. LC-MS grade isopropanol was infused into the REIMS interface at 100 $\mu L.min^{-1}$ during the measurements.

REIMS data was processed using ProGenesis Bridge (Waters) which splits sample files into new files with each individual replicate and subtracts the baseline of ambient air. Masses were aligned to the m/z for fatty acid C18:1 $[M-H]^-$ ion (281.2481) which was one of the major ions present in all samples. Data were then processed using ProGenesis QI (Waters) to normalise the data based on overall intensity, and search for the obtained high-resolution masses in the Human Metabolome Database (http://www.hmdb.ca) and Lipid Maps database (www.lipidmaps.org) with 5 ppm mass error. These database-based identifications can only be tentative as compound identification based on high resolution mass alone and only meets level 2 criteria for identification in metabolomics (Sumner et al., 2007). The identification of the compounds cannot be considered to be strong, yet are useful for understanding what compound classes are impacted by different meat aging methods and time. Here we have reported the m/z values for the significant compounds and discussed those compounds which have been identified and are known to be related to mammalian metabolism. In several cases there are many potential identifications, especially in the case of lipids where there are many

isomers. In such cases we have focused on the lipid class rather than identification of individual lipids.

2.4 Statistical analysis

REIMS data analysis was carried out to determine (1) the effect of ageing time (0, 7 and 21 days); (2) the effect of stepwise-ageing (T1 *vs.* T2, 21 days); (3) the effect of air velocities (T2-T4, 7 days); (4) the interactions of air velocities with stepwise-ageing (T1-T4, 21 days); and (5) the effect of trimming from three sampling locations.

The normalised abundance of the identified ions (1705 ions) produced from the technical replicates were combined into an average abundance for each sample. Differences between ageing treatments and sampling locations were determined using a combination of multivariate and univariate statistics on the identified ions (total 1705 ions). Principal Component Analysis (PCA), an unsupervised multivariate analysis tool, was used to determine if there was any underlying structure in the data that was not related to the research question (e.g. due to the analysis). Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA), a supervised multivariate analysis tool was used to determine the differences between different ageing treatments and times as this allows the determination of those molecular features which differ the most between treatments. Both PCA and OPLS-DA were done using SIMCA 16 (Umetrics, Umeå, Sweden). For OPLS-DA, R^2 (cumulative) and Q^2 (cumulative) scores were used to describe the robustness and accuracy of the present statistical model. R^2 describes the overall variation explained by all components in the model and Q^2 is the measure of robustness of the prediction. OPLS-DA models were of interest if the Q^2 score for predictability was > 0.2. A model with Q^2 score > 0.5 was considered as a good prediction of clusters (Broadhurst & Kell, 2006). Data were scaled to unit variance before OPLS-DA modelling to give all features equal weight in the model irrespective of their abundance.

The normalised mass intensities between treatments were compared using one-way ANOVA with t-test to separate the means at $P < 0.05$. To account for false positive results due to the high number of variables detected, it was assumed that of the results 5 % would have $P < 0.05$ by chance. Of the m/z values that were $P < 0.05$, 5 % of the total number of ions detected were removed (for example, if 1000 ions were detected, and 100 had $P < 0.05$, only the 50 with the lowest P values were considered to be different). Due to the high number of variables that differed between groups, we focused on two groups of ions: (1) dominant metabolites with highest average abundance (minimum threshold = 100); (2) high fold change metabolites with highest (> 1.1) and lowest (< 0.9) fold changes compared to corresponding treatment.

3 Results

A total of 1705 ions were detected and tentatively identified from dry-aged beef samples using REIMS. Thus, the current study focused on the ions which have significant ($P < 0.05$) variance between treatments with greatest fold change and abundance in the sample matrix. Some of the high molecular weight ions (> m/z 600), mainly lipids (Supplementary Figure 1), have only been assigned to one or more lipid classes due to the large number of potential isobaric and isomeric lipids associated with a single high-resolution m/z value. As REIMS does not include any chromatographic separation and nor is there the possibility to do MS/MS fragmentation during standard REIMS operation, it was not possible to further identify these ions. Thus, we grouped all the phospholipids into one class, as shown in Table 1-4. It should be noted that for the purposes of identifying markers related to meat processing, compound identification is not necessary. However, this is an important step for understanding the underlying biological reasons for the changes.

3.1 Effect of ageing methods (straight-dry-ageing *vs*. stepwise-ageing)

There was no clear clustering observed between ageing treatments using PCA modelling, and no apparent systematic trend due to run order or batch (data not shown). In-bag stepwise-ageing and straight-dry-ageing had clearly different metabolite profiles based on OPLS-DA modelling (Figure 1), with a Q^2 value of 0.85 suggesting that the naive model was robust with good predictability. Around 41% of metabolites differed significantly between the two ageing treatments. Out of those identified metabolites, 194 metabolites (approx. 11.38%) were significantly more abundant in stepwise-aged beef. In the straight-dry-aged beef, approximately 498 metabolites (approx. 29.21%) were significantly more abundant, with the dominant metabolite ions tentatively identified as amino acids, dipeptides, amines and metabolites produced from glycerophospholipid metabolism (Table 1). The fold changes of these metabolites over stepwise-ageing were around 1.2-1.3 except for glutamic acid (1.80, m/z 128.0, $[M\text{-}H_2O\text{-}H]^-$) and tyramine (1.41, m/z 410.3, $[3M\text{-}H]^-$) in straight-dry-aged beef. Amines and glycerophospholipids were the predominant metabolites in straight-dry-aged beef with significant changes (fold change 1.4-1.8, $P < 0.05$) compared to those stepwise-aged equivalents. On the other hand, the dominant metabolites with significant changes (fold change > 2.0) in stepwise-aged beef were low molecular weight ions (< m/z 400, Table 1).

There was no significant ($P > 0.05$) difference between the three air velocities in the stepwise treatment after 7 days of dry-ageing (T2-T4, data not shown). Further comparison between the two ageing methods was carried out by identifying the recurrent metabolites detected across the three stepwise-ageing treatments (T2-T4), which significantly differed from straight-dry-ageing method (T1). There were 5 metabolites (m/z 161.0, 189.1, 221.1, 340.2 and 365.2) consistently more abundant ($P < 0.05$) in stepwise-aged beef regardless of air velocities compared to the straight-dry-aged equivalents (Table 1). Higher level of

asparagine-lysine dipeptide (m/z 281.1, $[M+Na-2H]^-$) was also detected in stepwise-aged beef compared to straight-dry-aged counterpart. On the other hand, straight-dry-ageing resulted in consistently increased content of larger ions (> m/z 500) than stepwise-ageing (T2-T4), including m/z 369.2, 869.6, 893.6, 1201.8 and 1290.9. These ions were putatively identified as metabolites produced from lipid metabolism including glycerophospholipids and sphingolipids.

3.2 Effect of ageing time (0, 7 & 21 days)

A good predictive model with Q^2 value of 0.53 was obtained for differentiating between three ageing time points regardless of the ageing methods (Figure 2). Lean beef samples aged for 21 days were clearly separated from the unaged (0 day) and dry-aged for 7 days.

3.2.1 0 *vs.* 7 days dry-ageing

There were 9 recurrent metabolites detected in beef samples following 7 days of in-bag dry-ageing and these differed significantly ($P < 0.05$) from the unaged beef equivalents, regardless of air velocities. Dry ageing for 7 days resulted in significantly reduced level of some small ions (6 ions, < m/z 500) than unaged beef; these include fatty acid esters, sugar alcohols and long chain polyunsaturated fatty acids. Another three ions matching oleic acid (m/z 324.2, $[M+K-2H]^-$), lucidenic acid J (m/z 527.2, $[M+K-2H]^-$) and glycerophosphoinositol (m/z 895.6, $[M+Na-H]^-$) were also consistently more abundant in dry-aged beef (7 days, T1-T4) compared to its unaged equivalent.

3.2.2 0 *vs.* 21 days straight-dry-ageing

Meat composition was clearly different following 21 days of straight-dry-ageing in comparison to the equivalent unaged beef ($Q^2 = 0.95$), with 411 metabolites (24.11%) differing between the dry-aged and unaged meat. Out of these 411 metabolites, 164

compounds were more abundant at 21 days of straight-dry-ageing. As shown in Table 2, all the dominant metabolites decreased after 21 days of straight-dry-ageing period (fold change < 1.0). Straight-dry-ageing of lean beef for 21 days also produced significantly increased levels of low-medium molecular weight ions (< m/z 500), which are speculated to be dipeptides, free amino acids and fatty acids, and their derivatives. Another group of metabolites tentatively identified as amino acid derivatives, amines, heterocyclic fatty acids, and glycerophospholipids were found to be more abundant in unaged beef (0 day) with high fold changes compared to the dry-aged (Table 2).

3.2.3 0 *vs.* 21 days stepwise-ageing

The metabolic profile of stepwise-aged beef for 21 days differed significantly from the unaged equivalents with an OPLS-DA Q^2 value of 0.77, 0.71 and 0.71 for T2, T3 and T4, respectively. More than 40% of metabolites detected changed significantly after 21 days of stepwise-ageing process (690, 796 and 706 metabolites for T2-T4, respectively).

The dominant metabolites which changed significantly across the ageing time regardless of air velocities and which were found to be more abundant in unaged beef compared to those stepwise-aged for 21 days are shown in Table 3. There were 10 metabolites made of mainly medium-high molecular weight (> m/z 400-900) diglycerols and glycerophospholipids observed in unaged beef. Stepwise-ageing, on the other hand, produced significantly higher level of low-medium molecular weight metabolites compared to unaged equivalents.

3.3 Sampling locations

As shown in the PCA score plot (Figure 3a), the metabolic profile of intact dry-aged beef with untrimmed surface separated from the trimmed equivalent and from the trimmings (Q^2 = 0.40). This was further validated by an OPLS-DA model with three sampling locations

(Figure 3b) and acceptable prediction (Q^2 = 0.40). Stronger predictive models were generated when untrimmed meat surface was compared to the trimmings (Q^2 = 0.94) and the trimmed meat equivalent (Q^2 = 0.73). The modelling between trimmed meat and the trimmings resulted in a negative Q^2 value (Q^2 = -0.12) suggesting no significant difference existed between these two sampling locations.

More than 50% of metabolites differed significantly ($P < 0.05$) between untrimmed surface, trimmings (963 metabolites, 56.48%) and trimmed centre of the meat (1074 metabolites, 62.99%). The dominant metabolites which were also significantly ($P < 0.05$) more abundant in untrimmed surface meat mainly consisted of low-medium molecular weight ion, such as amino acids, dipeptides, amines, fatty alcohols, and aldehydes (Table 4). The medium-high molecular weight metabolites (mainly glycerophospholipids) were present at higher levels in trimmings and trimmed meat centre compared to untrimmed surface meat.

4 Discussion

4.1 Ageing methods

In this study, stepwise ageing was carried out by combining in-bag dry-ageing for 7 days followed by wet-ageing for 14 days. This method of drying may differ in proteolytic pathway with its straight dry-ageing equivalent. Three recurrent ions (m/z 245.0, 283.1 and 299.0) matching dipeptides were significantly more abundant in stepwise-aged beef for 21 days compared to straight-dry-aged equivalents (Table 1). This group of ions was also observed to increase after 21 days of straight-dry-aging (Table 2), suggesting that some of the primary proteolytic pathways by endogenous proteases (mainly calpain and calpastatin) is likely to be similar between the two ageing methods (Kim et al., 2018; Zhang et al., 2020).

Another ion of interest is m/z 201.0 ($[M-Na-2H]^-$) which was tentatively identified as a hexose. Many monosaccharides such as glucose, mannose, fructose or galactose have the

same molecular weight and cannot be conclusively identified using REIMS (Table 1). Post-mortem glycolysis within the first 24-48 hrs of slaughter is the primary biochemical pathway to produce sugar-related metabolites such as hexoses and trioses (Matarneh, England, Scheffler, & Gerrard, 2017). The significant increase in hexoses after 7 days of ageing could be associated with the enzymatic hydrolysis of glycoproteins (Koutsidis et al., 2008). Hexoses (glucose and mannose) have been reported previously by Kim et al. (2016) in aged beef regardless of ageing methods (wet-/dry-ageing). In this study, slightly different biochemical reactions may have occurred during the stepwise ageing compared to the straight-dry-ageing as discussed above, consequently resulting in the variations in the metabolic profiles between the two ageing methods. Further analysis using chromatography is needed to validate this finding.

4.2 Ageing time

Ageing time has been suggested to be one of the essential factors in the tenderisation and flavour development of meat (Kim et al., 2018; Zhang et al., 2020). Enough ageing time should be allowed for biochemical reactions to occur to convert meat to a "well-aged" product. In the current study, more ions identified as free amino acid valine (m/z 98.1, $[M\text{-}H_2O\text{-}H]^-$) and six dipeptides (ion mass of m/z 245.0, 281.1, 283.1, 299.0, 352.2 and 545.3, Table 2) detected in lean beef after 21 days of straight-dry-ageing. The significant increase of free amino acids (including valine) with ageing time up to 21 days (Zhang et al., 2020) or 28 days has also been reported (Kim et al., 2016; Lee et al., 2019).

It is well recognised that free amino acids and peptides are produced from enzymatic proteolysis during post-mortem ageing (Fu, Young, & Therkildsen, 2017; Nishimura, 1998). A post-mortem ageing period of around 3-4 weeks has been suggested to be necessary to significantly improve meat flavour (Campbell et al., 2001; Kim et al., 2018; Watanabe et al., 2015). This could be explained by the enzymatic proteolysis at refrigerated temperature (0-

4 °C) being slow (Bechtel & Parrish, 1983), and consequently requiring a longer time to produce significant amount of taste active metabolites and flavour precursors that contribute towards unique aged flavour (Kim et al., 2018). Ageing time of over 7 days has been suggested to produce significantly higher level of water-soluble flavour precursors including free amino acids and reducing sugars (Koutsidis et al., 2008; Zhang et al., 2020). In this study, no significant change of these flavour precursors following 7 days of in-bag dry-ageing has also been observed. Therefore, a short period of ageing time for 7-10 days could have produced quality acceptable dry-aged products, but mainly due to the improvement of tenderness as suggested in our previous study (Zhang et al., 2019). A longer period of ageing time of up to 3-4 weeks will produce premium niche products with the 'well-aged' signature including significantly more abundant free amino acids and small peptides being produced as detected by REIMS in the current study.

4.3 Air velocities

Increase of air velocities during traditional out-of-bag dry-ageing process has been suggested to result in different microbial compositions (mainly mould and yeast) and consequently modify flavour (Lee et al., 2019). However, our previous work has shown that increase of air velocity had not affected the quality, sensory acceptability (Zhang et al., 2019) and oxidative stability (Zhang et al., 2020) of in-bag dry-aged lean beef. The REIMS metabolic fingerprints where no impact of air velocity has been observed, support our previous findings. The use of water-permeable ageing bags for dry-ageing process protects the meat from microbial contamination by the surroundings therefore limits the growth of microorganisms. In-bag dry-ageing of beef has been reported to enhance the proliferation of yeast while having no impact on moulds, regardless of air velocities. Thus, the role of air velocity in the biomolecular changes of in-bag dry-aged beef is different from those out-of-bag regime.

4.4 Sampling locations

In this study, three different sampling locations were examined on intact straight dry-aged lean beef after 21 days of ageing, to determine the impact of trimming practice on the metabolic profile of dry-aged beef. Straight-dry-ageing for 21 days resulted in significantly higher levels of small ions, such as amino acids (glutamic acid, m/z 128.0) and dipeptides including (m/z 127.1, 205.1 and 830.4) on the untrimmed meat surface compared to the sampling locations from trimmings and trimmed meat centre (Table 4). Glutamic acid was one of the predominant metabolites detected on the untrimmed meat surface with about 4-fold higher than the trimmings and meat centre. The increase of glutamic acid on meat surface could be due to be the proteolytic reaction resulting from proliferation of yeast on the untrimmed meat surface (Li et al., 2014; Li, Babol, Wallby, & Lundström, 2013; Zhang et al., 2019). This hypothesis is further supported by the more abundant (fold change > 1.10) metabolite ions observed on the trimmings over the meat centre, including putative dipeptides such as alanine-glycine (m/z 127.1, $[M\text{-}H_2O\text{-}H]^-$), and hydroxyproline-proline (m/z 209.1, $[M\text{-}H_2O\text{-}H]^-$). Therefore, post dry-ageing trimming practice could result in loss of some biomolecular signatures for dry-ageing process such as small peptides and free amino acids which could be contributing to the distinct flavour of dry-aged meat.

Post-ageing trimming is a common practice in manufacturing dry-aged products using traditional out-of-bag process to remove discolouration, dehydration and microbial contamination, which mainly take place on the surface (Kim et al., 2018). These are not visually appealing to consumers and can also cause food safety issues. In this study, in-bag ageing process (i.e. stepwise-ageing) was developed using water-permeable ageing bags for dry-ageing and vacuum barrier bags for the wet-ageing. The use of ageing bag accelerates moisture evaporation and prevents microbial contamination (Ahnström, Seyfert, Hunt, & Johnson, 2006; Li et al., 2013; Zhang et al., 2019). The moisture difference between the

surface and the centre of dry-aged meat is equilibrated during the extended wet-ageing process thereby minimising the detrimental effect of surface dehydration associated with traditional out-of-bag dry-ageing process. Thus, removing the need for trimming the surface of the dry aged meat.

4.5 REIMS as a novel tool

The findings of the current study have demonstrated that REIMS can successfully differentiate the impact of different ageing treatments, ageing times and sampling locations on the metabolic profile of lean bull beef. As an exploratory approach, our results found that different dry-ageing regimes led to very different molecular fingerprints. To summarise, increase of dry-ageing time produced more small sized ions (< m/z 500) which consists of dipeptides, amino acids, fatty alcohol and organic acids. Production of small sized ions were also more evident on the surface/crust of dry-aged beef compared to the meat centre. Biomolecular changes by straight-dry-ageing resulted in more large sized ions, which were produced from phospholipid metabolism (e.g. oxidation) whereas for the stepwise ageing, 99% of the significant increase was driven by small sized ions.

To date, most assessments of dry-ageing methods and processes have used standard meat quality measures including sensory testing, measurements of pH, proximate content, instrumental colour and texture analysis. We have found previously that these were insufficient to differentiate between ageing methods (Zhang et al., 2019, 2020). In this study, REIMS results differed markedly between the different dry-ageing methods and has given some insights into some of the biological differences that may influence final dry-aged meat quality. As a relatively new metabolic fingerprinting technique in the area of food science, REIMS can perform close to real time profiling of thousands of metabolite ions to distinguish the samples based on their inherent properties and external processing treatments (Balog et al., 2016; Black et al., 2019). As no sample preparation is necessary there is scope for REIMS

and similar technologies to be applied within the meat industry and meat research for daily routine sampling per carcass/cut as a quality control measure. For example, pilot studies on the use of REIMS to detect boar-taint in an abattoir have been successfully carried out (Verplanken et al., 2017). Compared to chromatography-mass spectrometry methods (GC-MS and LC-MS) which are commonly applied for metabolic study of meat, REIMS has limitations of not being suitable for quantitative detection and real time qualification of identified molecules due to the difficulty in consistently introducing standard or reference molecules to meat samples. Further studies are required to establish the relationship between REIMS fingerprinting and conventional omics profiling (e.g. lipidomics and metabolomics) to validate current identified compounds.

5 Conclusion

REIMS was successfully applied in this study as a real time metabolic fingerprinting technique to detect the impact of different ageing treatments applied on lean beef samples. Ageing methods, ageing time and sampling locations have been clearly differentiated based on the 1705 tentatively identified metabolite ions. More abundant low molecular weight metabolites (dipeptides and amines) were associated with stepwise-ageing of lean beef compared to straight dry ageing for 21 days. Glutamic acid and several dipeptides could become candidate biomarkers for in-bag dry-aged lean beef. REIMS could also potentially be a suitable tool for identifying food fraud issues associated with dry-aged meat products when a definition for dry-aged meat is developed and widely accepted.

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References

Ahnström, M. L., Seyfert, M., Hunt, M. C., & Johnson, D. E. (2006). Dry aging of beef in a bag highly permeable to water vapour. *Meat Science, 73*(4), 674-679.

Balog, J., Perenyi, D., Guallar-Hoyas, C., Egri, A., Pringle, S. D., Stead, S., Chevallier, O. P., Elliott, C. T., & Takats, Z. (2016). Identification of the species of origin for meat products by rapid evaporative ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 64*(23), 4793-4800.

Bechtel, P. J., & Parrish, F. C. (1983). Effects of postmortem storage and temperature on muscle protein degradation: Analysis by SDS gel electrophoresis. *Journal of Food Science, 48*(1), 294-295.

Black, C., Chevallier, O. P., Cooper, K. M., Haughey, S. A., Balog, J., Takats, Z., Elliott, C. T., & Cavin, C. (2019). Rapid detection and specific identification of offals within minced beef samples utilising ambient mass spectrometry. *Scientific Reports, 9*(1), 6295.

Broadhurst, D. I., & Kell, D. B. (2006). Statistical strategies for avoiding false discoveries in metabolomics and related experiments. *Metabolomics, 2*(4), 171-196.

Campbell, R. E., Hunt, M. C., Levis, P., & Chambers, E. (2001). Dry-Aging Effects on Palatability of Beef Longissimus Muscle. *Journal of Food Science, 66*(2), 196-199.

da Silva, A. C. M., de Oliveira Pena, P., Pflanzer, S. B., & da Silva do Nascimento, M. (2019). Effect of different dry aging temperatures on *Listeria innocua* as surrogate for *Listeria monocytogenes*. *Meat Science, 157*, 107884.

Ellis, D. I., Broadhurst, D., Kell, D. B., Rowland, J. J., & Goodacre, R. (2002). Rapid and quantitative detection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. *Appl. Environ. Microbiol., 68*(6), 2822-2828.

Fu, Y., Young, J. F., & Therkildsen, M. (2017). Bioactive peptides in beef: Endogenous generation through postmortem aging. *Meat Science, 123*, 134-142.

Iida, F., Miyazaki, Y., Tsuyuki, R., Kato, K., Egusa, A., Ogoshi, H., & Nishimura, T. (2016). Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. *Meat Science, 112*, 46-51.

Kim, Y. H. B., Kemp, R., & Samuelsson, L. M. (2016). Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. *Meat Science, 111*, 168-176.

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. *Meat Science, 144*, 74-90.

Koutsidis, G., Elmore, J., Oruna-Concha, M. J., Campo, M., Wood, J., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour. Part II: Effect of post-mortem conditioning. *Meat Science, 79*(2), 270-277.

Laster, M. A., Smith, R. D., Nicholson, K. L., Nicholson, J. D. W., Miller, R. K., Griffin, D. B., Harris, K. B., & Savell, J. W. (2008). Dry versus wet aging of beef: Retail cutting yields and consumer sensory attribute evaluations of steaks from ribeyes, strip loins, and top sirloins from two quality grade groups. *Meat Science, 80*(3), 795-804.

Lee, H. J., Choe, J., Kim, M., Kim, H. C., Yoon, J. W., Oh, S. W., & Jo, C. (2019). Role of moisture evaporation in the taste attributes of dry- and wet-aged beef determined by chemical and electronic tongue analyses. *Meat Science, 151*, 82-88.

Lee, H. J., Won Yoon, J., Kim, M., Oh, H., Yoon, Y., & Jo, C. (2019). Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. *Meat Science, 153*, 152-158.

Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J., & Lundström, K. (2014). A comparative study of beef quality after ageing *longissimus* muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. *Meat Science, 97*(4), 433-442.

Li, X., Babol, J., Wallby, A., & Lundström, K. (2013). Meat quality, microbiological status and consumer preference of beef *gluteus medius* aged in a dry ageing bag or vacuum. *Meat Science, 95*(2), 229-234.

Matarneh, S. K., England, E. M., Scheffler, T. L., & Gerrard, D. E. (2017). Chapter 5 - The conversion of muscle to meat. In F. Toldra´ (Ed.), *Lawrie´s Meat Science (Eighth Edition)* (pp. 159-185): Woodhead Publishing.

Nishimura, T. (1998). Mechanism involved in the improvement of meat taste during postmortem aging. *Food Science and Technology International, Tokyo, 4*(4), 241-249.

Nishimura, T., Rhue, M. R., Okitani, A., & Kato, H. (1988). Components contributing to the improvement of meat taste during storage. *Agricultural and Biological Chemistry, 52*(9), 2323-2330.

O'Quinn, T., Woerner, D., Engle, T., Chapman, P., Legako, J., Brooks, J., Belk, K. E., & Tatum, J. (2016). Identifying consumer preferences for specific beef flavor characteristics in relation to cattle production and postmortem processing parameters. *Meat Science, 112*, 90-102.

Piasentier, E., Valusso, R., Camin, F., & Versini, G. (2003). Stable isotope ratio analysis for authentication of lamb meat. *Meat Science, 64*(3), 239-247.

Ross, A., Brunius, C., Chevallier, O., Dervilly, G., Elliott, C., Guitton, Y., Prenni, J .E., Savolainen, O., Hemeryck, L., Vidkjær, N. H., Scollan, N., Stead, S. L., Zhang, R. & Vanhaecke, L. (2020, in press). Making complex measurements of meat composition fast: Application of rapid evaporative ionisation mass spectrometry to measuring meat quality and fraud. *Meat science*, 108033.

Savell, J. W. (2008). Dry-aging of beef: Executive summary. Retrieved 2019.6.19, from Center for Research and Knowledge Management. National Cattlemen's Beef Association. http://www.beefissuesquarterly.com/CMDocs/BeefResearch/PE_Executive_Summaries/Dry_Aging_of_Beef.pdf

Smith, R. D., Nicholson, K. L., Nicholson, J. D. W., Harris, K. B., Miller, R. K., Griffin, D. B., & Savell, J. W. (2008). Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. *Meat Science, 79*(4), 631-639.

Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., Fan, Teresa W. M., Fiehn, O., Goodacre, R., Griffin, J. L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A. N., Lindon, J. C., Marriott, P., Nicholls, A. W., Reily, M. D., Thaden, J. J., & Viant, M. R. (2007). Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics, 3*(3), 211-221.

Trivedi, D. K., Hollywood, K. A., Rattray, N. J., Ward, H., Trivedi, D. K., Greenwood, J., Ellis, D. I., & Goodacre, R. (2016). Meat, the metabolites: An integrated metabolite profiling and lipidomics approach for the detection of the adulteration of beef with pork. *Analyst, 141*(7), 2155-2164.

Verplanken, K., Stead, S., Jandova, R., Van Poucke, C., Claereboudt, J., Bussche, J. V., De Saeger, S., Takats, Z., Wauters, J., & Vanhaecke, L. (2017). Rapid evaporative ionization mass spectrometry for high-throughput screening in food analysis: The case of boar taint. *Talanta, 169*, 30-36.

Watanabe, A., Kamada, G., Imanari, M., Shiba, N., Yonai, M., & Muramoto, T. (2015). Effect of aging on volatile compounds in cooked beef. *Meat science, 107*, 12-19.

Zhang, R., Yoo, M. J. Y., & Farouk, M. M. (2019). Quality and acceptability of fresh and long-term frozen in-bag dry-aged lean bull beef. *Journal of Food Quality, 2019*, 1975264.

Zhang, R., Yoo, M. J. Y., & Farouk, M. M. (2020, in press). Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. *Food Chemistry*, 128601.

Table 1 Effect of dry-ageing methods on the metabolic profile of dry-aged lean bull beef striploins.

Dominant metabolites									High fo
m/z	Ion/ Adduct	Putative compounds/Lipid class	*Fold change	*P*-values		m/z	Ion/ Adduct	Putative compou	
	Straight-dry-ageing (T1) for 21 days								
27.1	1 [M-H_2O-H]$^-$	Alanine-glycine dipeptide	0.87	0.031		11.1	2 [M-H]$^-$	2-Amino-3,4-dim	
28.0	1 [M-H_2O-H]$^-$	Glutamic acid	0.55	0.00		41.1	2 [M-H_2O-H]$^-$	Cyclo (Leucyl- P	
10.3	4 [3M-H]$^-$	Tyramine	0.71	0.004		19.2	3 [M+Cl]$^-$	Retinoid	
67.3	4 [M-H_2O-H]$^-$	Fatty amides	0.80	0.028		20.2	3 [M-H]$^-$	Arginine phenyla	
53.3	4 [M+K-2H]$^-$	Sterols/secosteroid	0.82	0.008		89.2	3 [M-H]$^-$	Pre-putrebactin	
79.3	4 [M+Cl]$^-$		0.81	0.030		08.2	4 [M+K-2H]$^-$	Anandamide (22:	
81.3	4 [M+Cl]$^-$		0.83	0.010		40.3	4 [M-H]$^-$	N-arachidonoyl h	
93.3	4 [M+K-2H]$^-$			0.036					
36.3	4 [M-H_2O-H]$^-$	Phospholipids	0.83	0.029		48.2	4 [M+Cl]$^-$	Sterols	
01.5	7 [M-H]$^-$		0.81	0.045		17.6	7 [M+K-2H]$^-$		
49.5	7 [M+Cl]$^-$		0.77	0.043		19.4	7 [M+K-2H]$^-$	Phospholipids	
64.5	7 [M+K-2H]$^-$		0.75	0.028		28.5	8 [M-H]$^-$		
65.5	7 [M+Na-2H]$^-$		0.74	0.018		29.6	8 [M-H_2O-H]$^-$		

						8	[M+		
					72.6	K-2H]⁻			
Stepwise-ageing (T2, dry-aging for 7 days then wet ageing for 14 days)									
	1	[M+	2-Methyl-1-methylthio-2-	3.08	<		1	[M+	Hydroxy fatty aci
37.0	Na-2H]⁻	butene			0.001	39.0	Na-2H]⁻		
	1	[M-	Glycerol 3-phosphate	1.17	0		1	[M-	Thymine glycol
53.0	H_2O-H]⁻				.004	41.0	H_2O-H]⁻		
	1	[M+	3,4-Diethylthiophene	3.64	<		1	[M+	S-(2-Furanylmeth
61.0#	Na-2H]⁻				0.001	91.0	Na-2H]⁻		
	1	[M-	1,2-dihydrostilbene	2.57	<		2	[M+	2-Amino-3,4-dihy
63.1	H_2O-H]⁻				0.001	00.0	Na-2H]⁻		
	1	[M+	2-Hexylthiophene	2.[illegible]	<		2	[M+	Branched Fatty A
89.1#	Na-2H]⁻				0.001	23.1	Cl]⁻		
	2	[M+	Hexose	5.38	<		2	[M+	Lipoic acid
01.0	Na-2H]⁻				0.001	27.0	Na-2H]⁻		
	2	[M+	1-(2-Thienyl)-1-heptanone	3.06	<		2	[M-	Aspartate-cystein
17.1	Na-2H]⁻				0.001	35.0	H]⁻		
	2	[3M-	Glycinamide	3.97	<		2	[M+	Cysteinyl-cystein
21.1#	H]⁻				0.001	45.0	Na-2H]⁻		
	2	[M+	Methionine-proline dipeptide	4.75	<		2	[M+	2-Ethyl-1-hexano
83.1	K-2H]⁻				0.001	45.1	Cl]⁻		
	3	[M+	Arachidonoyl amine	1.46	0		2	[M+	Hydroxypropyl-n
40.2#	K-2H]⁻				.008	99.0	K-2H]⁻	Hydroxyproline	
							[M+		Aspartate-Methio
							Cl]⁻		
	3	[M+	Estrane steroid	1.29	0				
65.2#	Na-2H]⁻				.001				

*Fold change is calculated by the ratio of metabolite intensity between stepwise ageing and straight-dry ageing after 21 days of ageing.

The m/z with "#" means the recurrent ions were also detected in stepwise-ageing regardless of ageing chamber air velocities of 0.5, 1.5 and 2.5 $m.s^{-1}$.

Table 2 Effect of ageing time (0 *vs.* 21 days) on the metabolic profile of straight-dry-aged (T1) lean bull beef striploins.

m/z		Ion/Adduct	Putative compounds/Lipid class	*Fold change	*P*-values
	Dominant metabolites				
138.1	1	[M-H_2O-H]$^-$	3-Methylcrotonylglycine	0.70	0.018
250.1	2	[M+Cl]$^-$	N-Nonanoylglycine	0.46	0.010
344.1	3	[M+Cl]$^-$	Lysine-tyrosine dipeptide	0.69	< 0.001
455.2	4	[M-H]$^-$	Phytyl diphosphate	0.62	< 0.001
327.3	5	[M+K-2H]$^-$	N-oleyl-alanyl-histidine	0.43	0.001
518.4	5	[M-H_2O-H]$^-$	Phospholipids	0.72	0.001
				0.84	
598.3	5	[M+Cl]$^-$		0.70	0.022
				0.69	
708.4	7	[M+Cl]$^-$		0.65	0.006
				0.68	
738.5	7	[M-H]$^-$			0.018
		[M+Cl]$^-$		0.74	
763.5	7	[M+K-2H]$^-$		0.66	< 0.001
773.4	8	[M+Cl]$^-$			0.019
1066.6	1	[2M+H ac-H]$^-$			0.010
1165.8	1				< 0.001
	Abundant at 21 days				

9	[M-H_2O-H]$^-$	Valine	1.70	0.005	8.1
1	[M-H_2O-H]$^-$	Proline betaine	1.50	0.011	24.1
1	[M+Cl]$^-$	Fatty alcohol	1.73	0.005	35.1
2	[M+Na-2H]$^-$	Cysteinyl-cysteine dipeptide	1.52	0.008	45.0
2	[M+Na-2H]$^-$	Asparagine-lysine dipeptide	2.07	0.012	81.1
2	[M+K-2H]$^-$	Methionine-proline dipeptide	1.90	0.006	83.1
2	[M+K-2H]$^-$	Hydroxyproline-methionine dipeptide	2.73	0.003	99.0
	[M+Cl]$^-$	Aspartate-methionine dipeptide			
3	[2M-H]$^-$	Proline- or lysine-derived Maillard product	1.31	0.036	53.2
4	[M-H_2O-H]$^-$	2'-Apo-beta-carotenal	2.26	0.011	89.4
5	[2M+Hac-H]$^-$	Lysine-proline dipeptide	1.65	0.009	45.3
7	[M-H_2O-H]$^-$	Phospholipids	2.14 1.55	0.003	68.5
8	[M+K-2H]$^-$		1.33	0.011	43.6
9	[M+Cl]$^-$			0.007	57.7
Abundant at 0 day					
1	[M-H_2O-H]$^-$	Alanine-glycine dipeptide	0.37	0.005	27.1
1	[M+Cl]$^-$	1-(1-Pyrrolidinyl)-2-butanone/3-(1-Pyrrolidinyl)-2-butanone	0.03	< 0.001	76.1

300.1	3	[M+K-2H]$^-$	Hydroxy-alpha-sanshool	0.45	0.010
326.2	3	[M-H_2O-H]$^-$	3-hydroxyundecanoyl carnitine	0.21	0.001
504.3	5	[M+K-2H]$^-$	N-Arachidonoyl tyrosine	0.40	< 0.001
771.7	7	[M+Na-2H]$^-$	Triacylglycerol	0.25	< 0.001
531.4	5	[2M-H]$^-$	Heterocyclic fatty acid	0.19 0.18	0.001
839.6	8	[3M-H]$^-$			0.002
517.3	5	[M+Na-2H]$^-$	Phospholipids	0.41 0.45	0.002
751.5	7	[M+Cl]$^-$		0.35 0.39	0.011
761.5	7	[2M-H]$^-$		0.39	0.002
804.6	8	[M+Na-2H]$^-$			0.002
363.9	1	[2M+Hac-H]$^-$			0.003
377.9	1	[M-H_2O-H]$^-$	Cardiolipin	0.37 0.27	0.003
389.9	1	[M+K-2H]$^-$			0.004

*Fold change is calculated by the ratio of metabolite intensity between ageing time of 21 days and 0 day.

Table 3 Effect of ageing time (0 *vs.* 21 days) on the metabolic profile of stepwise-aged lean bull beef striploins.

m/z	Ion/Adduct	Putative compounds/Lipid class
Abundant at 21 days, P < 0.05		
89.0	$[M-H]^-$	Lactic acid
293.2	$[M+Cl]^-$	2-Acetyl-3,5,5,6,8,8-hexamethyl-5,6,7,8- tetrahydronaphthalene
363.3	$[M-H_2O-H]^-$	Oxidative derivatives of cholesterol
393.3	$[M+Cl]^-$	
365.2	$[M+Na-2H]^-$	Estrane steroid
388.3	$[M+K-2H]^-$	Anandamide (20:4, n-6)
410.3	$[3M-H]^-$	Tyramine
Abundant at 0 day, P < 0.05		
404.3	$[M+Cl]^-$	Macamid
715.5	$[M+Cl]^-$	Diacylglycerol
717.5	$[M-H_2O-H]^-$	Phospholipids
767.5	$[M+K-2H]^-$	
769.5	$[M+Cl]^-$	
771.6	$[2M+Hac-H]^-$	
845.6	$[M-H_2O-H]^-$	
863.6	$[3M-H]^-$	
864.6	$[M-H]^-$	
865.6	$[M+Na-2H]^-$	

Table 4 Effect of sampling locations (surface of meat, trimmings and meat centre) on the metabolic profile of in-bag dry-aged untrimmed lean bull beef striploins.

Dominant metabolites			High fold change metabolites		
m/z	**Ion /Adduct**	**Putative compounds/Lipid class**	**m/z**	**Ion/Adduct**	**Putative compounds/Lipid class**
	Abundant at surface meat, P < 0.05				
109.0	$[M-H_2O-H]^-$	4-amino-4-cyano-butanoic acid	101.0	$[M-H]^-$	Sulfite
		2-amino-4-cyano-butanoic acid			
127.1	$[M-H_2O-H]^-$	Alanine-glycine dipeptide	105.0	$[M-H]^-$	Glyceric acid
128.0	$[M-H_2O-H]^-$	Glutamic acid	105.0	$[M+Na-2H]^-$	3-Methyl-2-butenal
135.1	$[M+Cl]^-$	Fatty alcohol	179.0	$[M-H]^-$	Nicotinuric acid
181.1					
154.1	$[M-H]^-$	Histidine	205.1	$[M-H]^-$	Glycine-methionine dipeptide
141.1	$[M-H_2O-H]^-$	Cyclo(Leu-Phe)	221.1	$[M+Cl]^-$	3-oxodecanoic acid
154.2	$[M+K-2H]^-$	N-methyl arachidonoyl amine	222.1	$[M+Cl]^-$	7-oxo-8-amino-nonanoic acid
169.2	$[M+Cl]^-$	Anandamide (18:4, n-3)	226.1	$[M+Na-2H]^-$	1-(2,3-Dihydro-1H-pyrrolizin-5-yl)-1,4-pentanedione
115.2	$[M-H_2O-H]^-$	Hydroperoxy fatty acid	271.2	$[M-H]^-$	16-Hydroxy hexadecanoic acid
149.4	$[M+Na-2H]^-$	Phospholipids	263.2	$[M+Na-2H]^-$	11-Hydroxy-9,15,16-trioxooctadecanoate
303.5	$[M-H]^-$				

	[M-H]$^-$	Triacylglycerol		[M-H]$^-$	Lysine-methionine dipeptide
99.7			30.4		

Abundant in trimmings and meat centre, P < 0.05

	[M+Cl]$^-$	Octadecanol		[M-H]$^-$	Erythro-5-hydroxy-L-lysinium(1+)
05.3			88.3		
	[2M+Hac-H]$^-$	Xylene		[M+Na-2H]$^-$	Sphingomyelins(d18:1/12:0)
95.5			67.5		
	[M+Na-2H]$^-$	Sphingomyelins(d18:0/12:0)		[M+Na-2H]$^-$	PE-Cer(d14:2(4E,6E)/22:1(13Z))
69.5			05.5		
	[M-H_2O-H]$^-$	Diacylglycerol		[M-H_2O-H]$^-$	Phospholipids
71.5			68.5		
	[M+K-2H]$^-$	Cholesterol fatty acid ester		[M+Na-2H]$^-$	
99.5			07.5		
	[M+Na-2H]$^-$	Phospholipids		[M+K-2H]$^-$	
79.5			11.5		
	[M-H_2O-H]$^-$			[M+Cl]$^-$	
81.5			52.[illegible]		
	[M-H]$^-$			[M-H]$^-$	
83.5			61.5		
	[M-H_2O-H]$^-$			[M+Na-2H]$^-$	
85.5			15.6		
	[M+Na-2H]$^-$			[M-H_2O-H]$^-$	
09.5			16.6		
	[M+K-2H]$^-$			[M+Na-2H]$^-$	
13.5			18.6		
	[M-H]$^-$			[M-H]$^-$	
21.5			98.6		
	[M+Cl]$^-$			[M+Cl]$^-$	Cardiolipin
66.6			483.9		

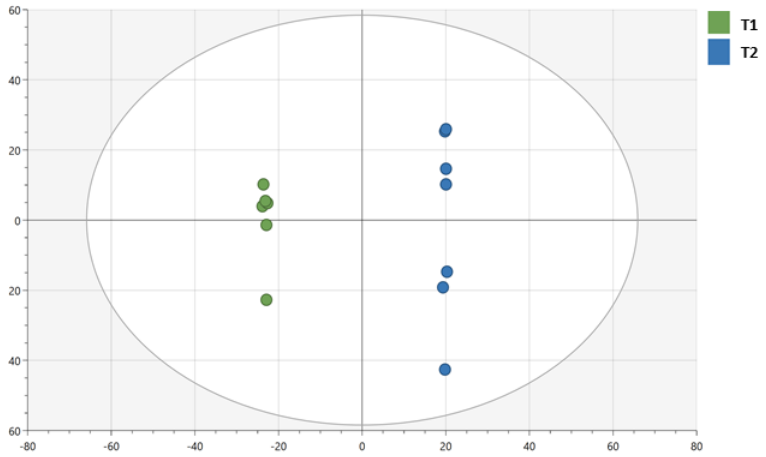

Figure 1 OPLS-DA score plot of the comparison between in-bag straight-dry-ageing (T1) and stepwise ageing (T2) ($R^2X = 0.79$, $Q^2 = 0.85$) of lean beef.

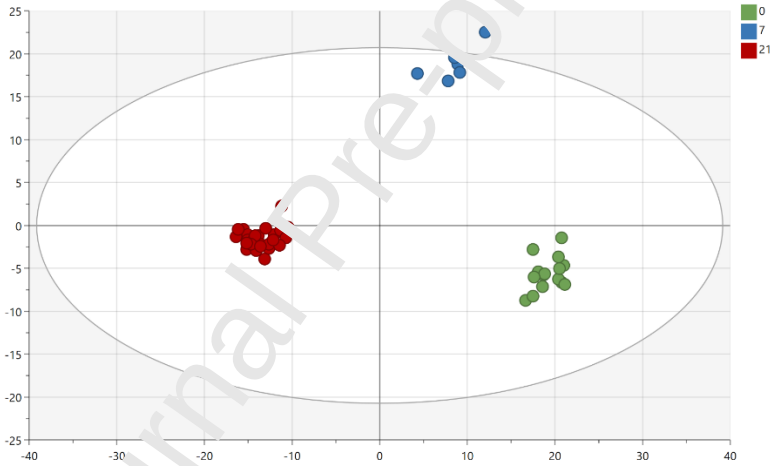

Figure 2 OPLS-DA score plot of the effect of ageing time (0, 7 and 21 days) on lean beef regardless of ageing methods (R^2X: 0.73, $Q^2 = 0.53$).

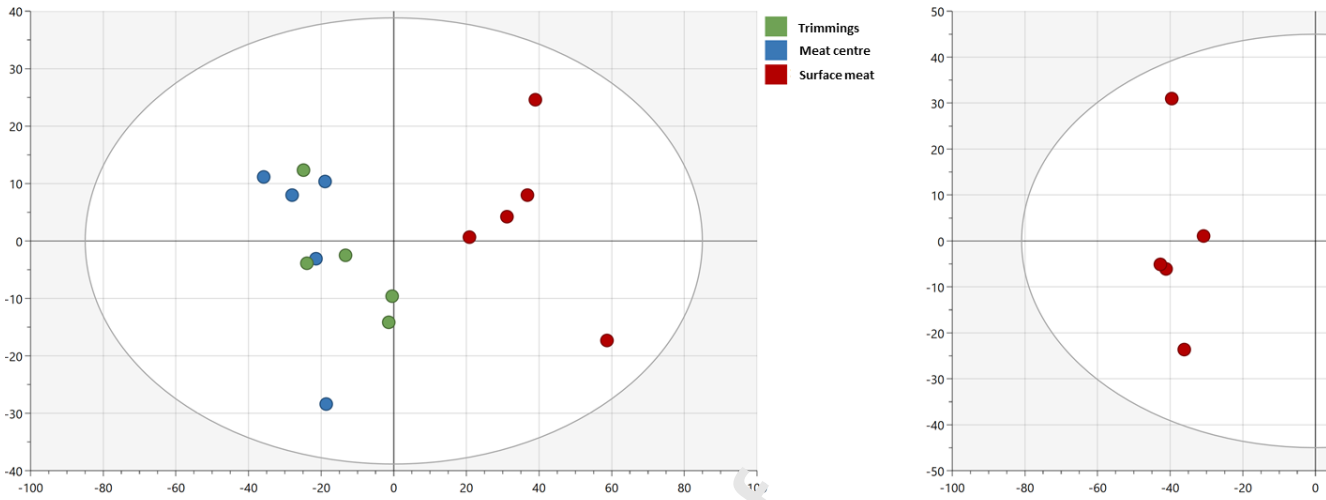

Figure 3 Effect of sampling locations (surface of untrimmed dry-aged lean beef, trimmings from dry-aged beef equivalent, and centre of a trimmed equivalent: a. PCA score plot of three sampling locations (R^2X = 0.62, Q^2 = 0.40); b. OPLS-DA score plot of three sampling locations (R^2X = 0.61, Q^2 = 0.40) on straight-dry-aged lean beef.

Author statement

Renyu Zhang: Conceptualization, investigation, resources, original draft; Alastair Ross: Methodology, resources, review & editing; **Michelle Yoo**: Supervision, review & editing; **Mustafa Farouk**: Supervision, review & editing

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: