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Beyond standard PSE testing: An exploratory study of bioimpedance as a marker for ham defects

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ABSTRACT

During post-mortem conversion from muscle to meat, diverse quality anomalies can emerge. Recent pork defects are often accompanied by deteriorating fibre structure. Here we investigate how bioimpedance response, an indicator of structural disintegration, can help in detecting quality defects. We, first, measured the relationship between standard meat quality variables (pH_u, CIELAB, drip loss) and bioimpedance (BI) response. To screen for defect-biomarkers that are linked to aberrant bioimpedance and physicochemical indicators of quality decline, we performed LC-MS/MS proteomic analysis on samples, classified with a multivariate-based separation into good versus poor quality. We found that BI correlated significantly with, e.g., colour and drip loss. Proteomics revealed eleven proteins to be unique for either, good or poor ham quality groups, and maybe linked to structural degradation. In all, our data supports a wider integration of BI testing in pork quality testing to assess structural disintegration, which can render ham unsuitable for, e.g., costly curing.

1. Introduction

Severe pork ham quality defects are currently reported for several European countries. Most quality issues have been found for producers of cooked and cured ham products. Ham defects can be present in up to 50% of processed hams causing great economic loss (Hugenschmidt et al., 2010; Théron et al., 2019). Ham defects are not limited to specific pig breeds or to certain pre- or post-slaughter management systems (transport, stunning and chilling) (Grandin, 1994; Rosenvold & Andersen, 2003). Rather, defects may be linked to multiple causal factors, which calls for large scale and reproducible defect mapping among meat producers. Yet, ham quality classification systems differ among producers and researchers, and objective, instrument-based detection methods that can directly assess structural damage are not yet marketed.

Pale soft exudative meat (PSE) is among the best-known hereditary pork defects (Adzitey & Nurul, 2011; Barbut et al., 2008). PSE is associated with a mutated calcium channel (ryanodine receptor) and a higher susceptibility to acute stress before slaughter (Trevisan & Brum, 2020). In PSE meat, accelerated anaerobic glycolysis and lactic acid accumulation triggers a rapid post-mortem pH drop. Low pH and high temperature after slaughter lead to muscle protein denaturation (Briskey, 1964). As a result, a loss of ordered cellular components can contribute to colour changes (paler meat), lack of firmness, and excessive drip loss. Another relevant pork defect that is also caused by a specific mutation is acid pork (AP). AP is characterized by high muscle glycogen content, which can cause very low ultimate pH values (Estrade, Vignon, & Monin, 1993). As with PSE, pH anomalies in AP contribute to pale discolouration and reduced water holding capacity (Le Roy et al., 2000). Both, PSE and AP meats are known to be associated with a single mutation. While the genetics of PSE and AP meat are well studied and effects on colour and drip loss (Hamilton, Mike Ellis, Miller, McKeith, & Parrett, 2000), PSE and, to a lesser extent also AP have not been completely eradicated (Vautier, Boulard, Bouyssière, Houix, & Minvielle, 2008).

Mapping of common pork defects usually relies on pH, colour, and drip loss measurements (Garrido, Pedauye, Banon, & Laencina, 1994). Typically, changes among these variables are found to be correlated, pointing to underlying physiological links (Adzitey & Nurul, 2011). The

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reduced water holding capacity is thought to be mainly caused by myofibrillar protein degradation, which in turn is affected by low pH, ionic strength, and oxidation of proteins (Huff-Lonergan & Lonergan, 2005). Increased paleness has been attributed to precipitation of sarcoplasmic proteins into extracellular space as well as unbound water that can reflect more light (Hughes, Clarke, Purslow, & Warner, 2020). Although pH, colour and drip loss are most commonly used for quality monitoring and sorting of meat cuts, there might be limitations concerning the accurate detection of other emerging pork defects with similarities to PSE and AP. Often such defects are called PSE-like meat, sometimes also destructured meat or PSE-zones (Eliášová et al., 2017; Laville et al., 2005; Théron et al., 2019). Yet, quality-defect classification systems based on common physicochemical data are not consistent, and often are specifically developed for loin, not ham cuts. On the other hand, established subjective sensory evaluation protocols for PSE-like ham require trained observers (IFIP, 2005; NPB, 1999) or might suffer from lower reproducibility. Instrument-based pork defect detection is currently developed for more robust meat quality monitoring, often using near-infrared spectroscopy, image analysis or bioelectrical instruments (Dixit et al., 2017; Eliášová et al., 2017; Zheng, Sun, & Zheng, 2006). Bioelectrical testing can involve simple conductance measurements (Antosik, TarczyÅ, Sieczkowska, & Zybert, 2022) or more advanced spectroscopic analyses based on a tissue's bioimpedance (BI) response. Instruments for BI measurements are widely used for clinical diagnostics, and to a lesser extent for food quality monitoring (Barsoukov & MacDonald, 2005). BI testing is rapid and can be performed with non-invasive or minimally invasive probes, which induce alternating electrical signals at different frequencies into the tissue and register the – usually – frequency dependent response.

In bioimpedance, the main variables are capacitance and resistance, which depend on changes in cell membrane integrity and on the distribution of extra- and intracellular fluids. Thus, for meat, bioimpedance measurements directly reflect structural damage to the cellular matrix as well as excessive drip channel formation (Egelandsdal et al., 2019; Kyle et al., 2004; Pliquett, Altmann, Pliquett, & Schoberlein, 2003). More specifically, bioimpedance is the property of a material to resist the flow of alternating electrical current, and hence, is dependent on the frequency of the applied electric current. Biological materials have both resistive (real part, R) and capacitive (imaginary part, X) properties. In our study we assessed bioimpedance by calculating the P_y parameter, an approach previously described for pork quality testing, including freeze damage and defects in raw pork (Abie et al., 2021; Pliquett et al., 2003). Yet, how BI-based quality testing is linked to common physicochemical quality features used to assess PSE-like pork is poorly understood.

To characterize molecular mechanisms of meat defects, both bioelectrical measurements and proteomic analysis can provide relevant information (Hou et al., 2020; Morey, Smith, Garner, & Cox, 2020). Proteomic research is used to potentially identify defect markers that can be predictive for meat quality traits (Purslow, Gagaoua, & Warner, 2021). What is more, functional gene ontology annotation (GO) can help understanding the biological functions that detected proteins are involved in. As a result, meat defect oriented molecular investigations are widely used to identify and describe biomarkers associated with meat quality issues that can be linked to myopathies, to excessive water loss or colour changes (Di Luca, Alessio, Hamill, & Mullen, 2013; Yang et al., 2018; Kuttappan et al., 2017b).

BI-based meat quality monitoring allows to assess microstructrual, cellular damage, a most severe symptom in common pork defects. However, potential links with more established quality tests are poorly investigated. As PSE-like pork quality defects typically affect multiple quality traits, including structural disentigration, we hypothesized that BI response is linked to changed pH_u, CIELAB colour coordinates and drip loss, and assessed potential correlations between bioelectrical measurements and standard quality measures. We then asked if multivariable-based selection – including BI – can reveal relevant proteomic changes in poor vs. good quality samples.

2. Materials and methods

2.1. Pork samples

Ham samples representing the *musculus semimembranosus* (SM) from finishing pigs (*Sus scrofa domestica*) were collected in December 2020 from a total of 84 animals, slaughtered in a Norwegian slaughterhouse on two different days. Each day, we collected 42 samples that came from 42 animals from the co-localized cutting plant. Pre-slaughter handling and slaughtering procedures were carried out in agreement with EC guidelines.

Chilled samples were collected at the meat plant, packed in boxes lined with plastic bags, and were transported without additional cooling by car (ca. 30 min) to a chilling room (4 °C), where samples were stored for all consecutive measurements.

All measurements were done on the SM. Two fixed locations, one lateral and one medial part of the muscle, were chosen (Supplementary Fig. 1S). All testing was done within a similar post-mortem time window (approximately 72 h post-mortem).

2.2. Physicochemical quality testing

The ultimate pH (pH_u) was measured using a pH 3110 Set 2 (WTW, Germany), equipped with a spear head pH electrode (BlueLine 21 pHT, SI Analytics, Germany). The electrode was calibrated against fresh buffers with pH of 4.0 and 7.0. Drip loss testing (2 measurements/slice) was performed with the EZ-DripLoss system (Otto, Roehe, Looft, Thoelking, & Kalm, 2004; Rasmussen & Andersson, 1996). The metal stand holding the EZ-DripLoss cups (DMRI, Denmark) was placed at 4 °C the day before sampling. The samples were cut using a circular sharp knife, placed in cups, and closed with a lid. After storage at 4–6 °C for three days, the samples were removed from the cups, and the cups containing the meat juice were weighed. Colour coordinates were measured using a Konica Minolta Chroma Meter CR-400 (Konica Minolta Sensing INC, Tokyo, Japan) with an illuminant D65 unit (Daylight, colour temperature 6504 K), a 2^o standard observer and a 0.8 cm port/viewing area. Meat colour was determined after exposing the samples to air for at least 1 h. For calibration a white ceramic calibration cap CR – A43 was used. The light source was a pulsed xenon lamp (Konica Minolta Sensing INC, Tokyo, Japan).

2.3. Bioimpedance

The bioimpedance response of meat was measured using a Zurich Instruments MFIA impedance analyser (Zurich Instruments AG, Zürich, Switzerland). The frequency range was 10 Hz to 1 MHz with 40 distinct frequencies and an applied voltage of 300 mV rms. A tetrapolar electrode setup was used (Egelandsdal et al., 2019). The stainless-steel needle electrodes had diameters of 2 mm and a length of 12 mm with 18 mm distance between the middle, voltage pick-up electrodes. Shielded cables with a length of 1 m connected the MFIA device to the electrode socket. Two readings were recorded from each location of the electrode. The electrode was cleaned after every measurement.

Bioimpedance (Z) is defined as the ratio of a voltage (V) over the electric current (I) that it generates (Z = V/I), and it represents the total opposition of the flow of electric current in a medium. This property depends on the characteristics of the biological medium through which the current flows and the applied signal frequency. Generally, bioimpedance is a complex number with electric resistance (R) as the real part, and reactance (X) as the imaginary part, both expressed in Ohm (Ω).

Mathematically electrical impedance (Z) can be presented by the equation:

$$\boldsymbol{Z} = R + jX \tag{1}$$

where the real part R is an indication of the ability of the molecules to dissipate electrical energy from an electromagnetic field, and the imaginary part X to store it.

Bioimpedance spectroscopy provides a response data as a function of frequency and can be fitted with the Cole equation to give Cole parameters R_0 and R_∞ (Cole, 1940). Calculation of the P_y parameter is shown in Eq. 2, where R_0 and R_∞ are the electrical impedances at very low and very high frequencies, respectively. The ratio of R_0 to R_∞ is proportional to the ratio of extracellular water to total water content in the ham. Physically, the P_y is a monotonically increasing function of the cell volume fraction surrounded by intact cell membranes (Pliquett & Pliquett, 1999).

$$P_y = \frac{R_0 - R_\infty}{R_0} = 1 - \frac{R_\infty}{R_0} \quad (2)$$

According to (Pliquett et al., 2003) the P_y ranges between 0.85 and 0.95 for fresh meat, depending on the kind of meat, but may also decline post-mortem to very small values ($P_y < 10$).

2.4. Proteomic analysis

2.4.1. Sample selection

For proteomic analyses we aimed at selecting samples that represent extremes within a population exhibiting marked quality heterogeneity. To this end, a principal component analysis (PCA) was performed based on pH_u, lightness, drip loss and bioimpedance (P_y) variables. Three samples representing normal quality meat and another three samples representing poor quality meat were selected for subsequential proteomics analysis.

2.4.2. Protein extraction

Meat samples were defrosted, and aliquots (0.5 g), devoid of visible fat and connective tissue, were then dissected using sterile forceps. The aliquots were homogenized in 1 ml of 200 mM potassium phosphate buffer at pH 7.4 with a Precellysä homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France, settings: 5000–1×20–005 for 1–2 min), and finally centrifuged at 10000×*g* for 20 min. We collected the supernatant (sarcoplasmic protein fraction).

2.4.3. Determination of protein concentration

The total protein concentration of the meat samples was determined using a Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies, CA, USA). Different dilutions of samples were made using extraction solutions. The mean protein concentration of the dilutions was calculated from the measured values obtained from the calibration curve. The conversion of dilution values to stock was done manually using the equation provided in the manufacturer's protocol. We diluted all samples to μg/μl, and volumes of 10 μg total protein per sample were dried using a SpeedVac concentrator (Eppendorf, Hamburg, Germany) prior to digestion.

2.4.4. In-solution trypsin digestion of samples for proteomic extraction

The proteins in meat samples were digested in-solution (Carvalho, Delgado, Madruga, & Estévez, 2021; Fæste et al., 2016; Fuente-García, Sentandreu, Aldai, Oliván, & Sentandreu, 2021; Kuttappan et al., 2017a). The aliquots (10 μg of total protein/sample) were re-dissolved in 100 μl of reaction buffer (50 mM NH4HCO3, pH 7.8), and reduced using 5 μl 200 mM Dithiothreitol (DTT) in reaction buffer (Sigma-Aldrich, St. Louis, MO, USA) for 60 min at 37 °C. The protein mixture was allowed to cool to room temperature and spun gently to collect the condensation. The samples were then alkylated with 15 μl 200 mM Iodoacetamide (Sigma-Aldrich, St. Louis, MO, USA), and incubated dark at 37 °C for 60 min. Alkylation was inhibited by adding 20 μl of 200 mM DTT into the sample mix, gentle vortexing and incubation for 30 min at 37 °C. Samples were then diluted with 100 μl of reaction buffer. The diluted proteins were digested with Trypsin (PierceÔ Trypsin Protease-MS Grade, Thermo Scientific, Waltham, Massachusetts, USA) such that the ratio of enzyme to protein in each sample was between 1:10 and 1:20 (*w*/w). The samples were incubated overnight in a thermomixer (Eppendorf AG, Hamburg, Germany) with a shaking speed of 800 rpm at 37 °C. After incubation, samples were concentrated by centrifugation (Eppendorf Centrifuge, Hamburg, Germany) for 5 min at 13,000 ×*g* and 4 °C, followed by drying in a SpeedVac concentrator (V-AQ, 45 °C) and re-dissolving in 15 μl of 1% formic acid (Merck, Darmstadt, Germany). After sonication for 30 s, and centrifugation for 5 min at 13,000 ×*g*, the samples were stored at −20 °C until the clean-up was performed. Lastly, we desalted the samples using C18 ZipTipâ pipette tips with tip size 10 (Merck Millipore Ltd., Carrigtwohill, Cork, Ireland). This was followed by centrifugation (10 min at 13,000×*g*) and drying with the SpeedVac concentrator. Samples were re-dissolved in 15 μl of 0.1% formic acid and the peptide concentration was checked using a Nanodrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). The dissolved samples were diluted to 200 ng/ μl of peptide concentration per sample using 0.1% formic acid, centrifuged for 10 min at 13,000×*g* and transferred to mass spectrometry vials. Vials were stored in a fridge for immediate analysis or kept at −20 °C for longer term storage.

2.4.5. Untargeted proteomic analysis by LC-MS/MS

Peptide samples were injected into a trap column (Acclaim PepMap100, C18, 5 μm, 100 Å, 300 μm i.d. x 5 mm, Thermo Scientific), and backflushed into a 50 cm analytical column (Acclaim PepMap RSLC C18, 2 μm, 100 Å, 75 μm i.d., Thermo Scientific). The following solutions were used: solution A [0.1% (*v*/v) formic acid], 4% solution B [80% (v/v) ACN, 0.1% (v/v) formic acid]. Peptide elution was performed with a flow rate of 300 nl/min using a 70 min method. The following gradients were applied: from 3.2 to 10% B within 3 min, 10 to 35% B within 94 min and 35 to 60% B within 3 min. This was followed by a wash at 80% B for 5 min and a subsequent equilibration for 15 min at 4% B. The Q-Exactive mass spectrometer was used in data-dependent acquisition (DDA) mode using a Top10 DDA method, where acquisition alternates between orbitrap-MS and higher-energy collisional dissociation (HCD) orbitrap-MS/MS acquisition of the 10 most intense precursor ions. Only 2–5 charge states were designated for fragmentation. The normalized collision energy (NCE) was set to 28. The selected precursor ions were excluded for repeated fragmentation for 20 s. The resolution was set to $R = 70{,}000$ and $R = 17{,}500$ for MS and MS/MS, respectively. Automatic gain control values were set to 3×106 and 5×104 for MS and MSMS, respectively, with a maximum injection time of 100 and 128 ms.

2.4.6. Identification and functional annotations of proteins unique for each quality group

We analysed all MS/MS samples using Mascot (Matrix Science, London, UK; version 2.6.1). Mascot search was performed assuming the digestion enzyme trypsin, with a fragment ion mass tolerance of 0.020 Da, and a parent ion tolerance of 10.0 PPM. Carbamidomethyl of cysteine was specified in Mascot as a fixed modification. Deamination of asparagine and glutamine, oxidation of methionine, and acetylation of the N-terminus were specified in Mascot as variable modifications.

Given the relatively low sample number, we have chosen a conservative approach for identifying proteins that are unique for either good- or poor-quality samples. Specifically, we list only proteins as unique (compare Table 2), when they were detected in all three samples of one but not in any sample of the other quality group. Proteins identified as good quality and poor quality samples were then subjected to Gene Ontology (GO) analysis. We used the ShinyGO tool (v0.75; http://bioinformatics.sdstate.edu/go/) for analysis of proteins that are found to be unique for either quality group.Venn diagrams were created using the online tool "Bioinformatics & Evolutionary Genomics" (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.5. Statistical analysis

Statistical analysis was performed using RStudio (Boston, USA). Analysis of potential links among quality parameters was conducted using the correlation coefficient (r). Correlation plots, Principal Component Analysis (PCA) plots and charts were used to visualize the results. Mean values and correlations between traditional meat quality parameters with P_y parameter were reported. Paired *t*-tests for pH_u, colour, drip loss and P_y values were performed to test if there were differences between lateral and medial locations in ham using Minitab version 19 (LLC, Pennsylvania, USA). Significance levels were $P = 0.05$, $P = 0.01$, $P < 0.001$ for all tests. Multiple regression analysis with Minitab allowed optimized model building with pH_u, drip loss, $L^*a^*b^*$ as x and P_y as the predicted, y-variable. The regression model was built using 2-way-interaction and quadratic fitting for the x-variables and step wise regression for predictor variable selection.

3. Results

3.1. Variations between tested locations

As muscle fibre degradation and dimension may not be homogenous within a single muscle, we first tested if location can affect quality measurements. We included two test locations, lateral and the medial SM (Supplementary Fig. 1S). For the two locations we obtained the same set of quality features, including BI response (P_y), as well as pH_u, CIELAB colour coordinates and drip loss.

Fig. 1 shows that all individual quality features we assessed at the two different locations show a strong positive correlation with one another (e.g., P_y lateral with P_y medial: $r = 0.84$, $P < 0.001$). In addition, Table 1 shows that location significantly affects all meat quality features, including P_y. Therefore, we chose only one specified location, the lateral SM, for all consecutive analyses.

3.2. Heterogeneity of quality features within the tested population

To ensure that bioimpedance tests are performed in a sample set with sufficient inter-individual quality differences, we first assessed the heterogeneity of the sample population with regards to standard meat quality parameters. Fig. 2 shows a relatively broad distribution of pH_u, lightness (L^*) and drip loss among the tested 84 ham samples. Further, while available meat defects classification systems are not entirely consistent, $pH_u < 5.5$ and $L^* > 55$, and drip loss >5% are often considered indicative of undesired pork quality. Based on these thresholds, we confirm that >39% of the entire sample population showed aberrant pH_u, L^* and drip loss values.

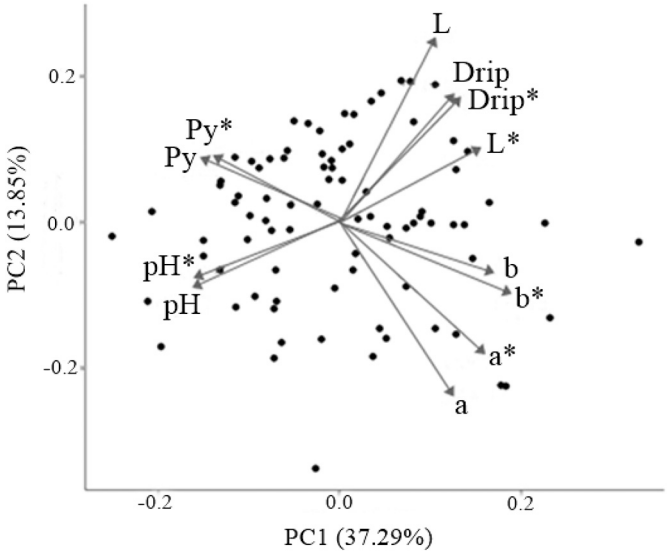

Fig. 1. Biplot of the PCA analysis of all variables (medial variables marked with *). Correlation r and *P*-values are as follows: P_y vs P_y*, $r = 0.84$, pH_u vs pH_u*, $r = 0.67$, L vs L^*, $r = 0.49$, b vs b^*, $r = 0.48$, a vs a^*, $r = 0.44$, drip loss vs drip loss*, $r = 0.57$, $P < 0.001$. 51.14% of variance is explained by PC1 and PC2. The magnitude of arrows indicates contribution to explaining the variability in a data set.

Table 1
Physicochemical and P_y measurements at the two test locations (mean value, standard deviation, and P-values; *t*-test).

	Lateral part* (84)		Medial part* (84)		
	Mean	S.D.	Mean	S.D.	P-value**
P_y	47.34	16.45	39.46	15.9	<0.01
pH_u	5.44	0.07	5.46	0.08	<0.05
Lightness (L^*)	56.87	3.89	54.01	3.63	<0.01
Redness (a^*)	6.99	1.92	9.27	1.95	<0.01
Yellowness (b^*)	8.09	2.08	8.62	2.10	<0.05
Drip loss (%)	5.44	1.60	5.05	2.15	=0.055

P_y – Bioimpedance parameter.
pH_u – Ultimate pH.
* Measured at the SM.
** $P < 0.05$ means a significant difference between muscle locations.

Bioimpedance, as a more direct indicator of tissue integrity was analysed for the same 84 ham cuts (lateral SM). As before (compare Fig. 2), we report marked inter-individual variation for both, the raw bioimpedance spectra (Fig. 3A), and for P_y, which was extracted from the individual spectra (Fig. 3B). Specifically, while all the meat samples showed dispersion (frequency dependence) within the full frequency range between 10 Hz to 1 MHz, we also observed inter-individual differences within bandwidths representing the low frequency α-dispersion (mHz–kHz; asterisk in Fig. 3A), and the higher frequency β-dispersion (1 kHz–1 MHz, arrowhead in Fig. 3A). However, differences were most pronounced for the β-dispersion, for which a 'flattened curve' typically indicates compromised cell and membrane integrity. Likewise, the parameter describing the steepness of β-dispersion, P_y (Fig. 3B), was observed to be low (<40) in >25% of the samples. This indicates compromised quality and cellular integrity in a sizable part of the sample population.

3.3. Correlation of P_y with other quality variables

PSE pork ham defects are typically described as a syndrome with multiple deterioration symptoms, which do correlate to some extent (compare Introduction). We therefore hypothesized that P_y, as an indicator of structural deterioration, should correlate with other key quality features of the defect syndrome. To this end, we calculated a Pearson Correlation coefficient matrix (Fig. 4) for the data shown in Figs. 2 and 3. Across the traditional physicochemical quality modalities, we found correlation strength to be very diverse. Correlations were strongest between the different colour measurements (e.g., L^* vs b^*, $r = 0.44$, b^* vs a^*, $r = 0.65$, $P < 0.001$). However, correlations among pH_u and colour with drip loss variables were typically between 0.2 and 0.4, ($P < 0.05$).

For the bioelectrical measurements we report moderate correlations for P_y vs. the colour variables a^* ('redness', r=-0.32, P<0.01) and b^* ('yellowness', $r = -0.45$, $P < 0.001$), as well as for P_y vs. driploss ($r = -0.31$, $P < 0.01$). Only weak, yet significant, correlations were found for P_y vs. pH_u as well as P_y vs. colour L^* (lightness). Adding to this, we carried out a step wise multiple regression model building with pH_u, drip loss and $L^*a^*b^*$ as predictors for P_y. The optimized model comprised three selected x-variables ($P_y = -342 + 14.84(L) - 3.322(b^*) - 2.51$(drip loss) $- 0.1276(L)^2$) and showed a higher prediction ($R^2 = 27.8\%$, $P < 0.01$) as compared to results from pairwise (Pearson) correlations. Together, correlation strengths of P_y with traditional quality paramters were moderate to weak, and comparable to those found also among pH_u,

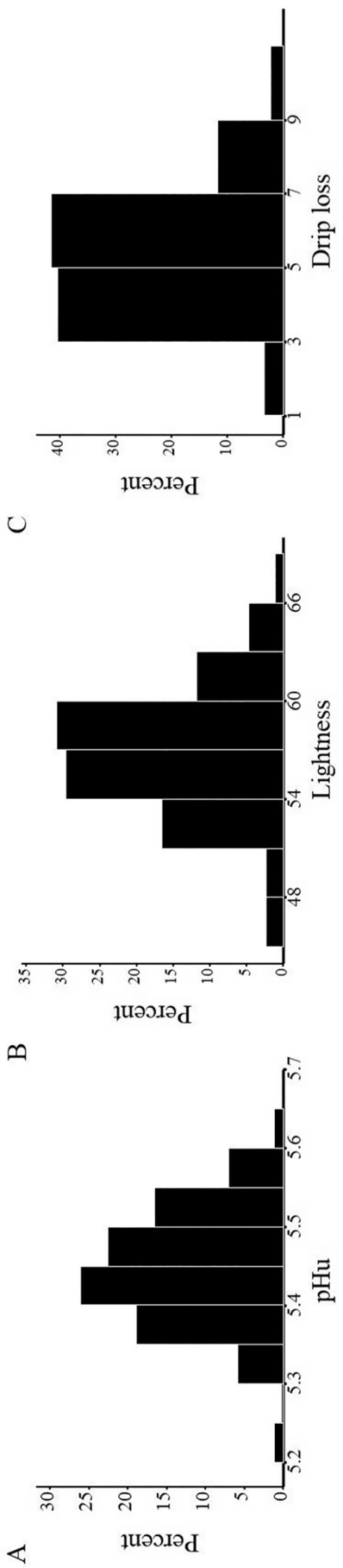

Fig. 2. Testing common quality parameters revealed a marked quality heterogeneity within the sample population ($N = 84$). A. Measured pH_u values ranged from $pH_u < 5.3$ to >5.6. B. Detected L^* values varied between 46.05 and 66.27. C. Drip loss ranged from 1.3% to 9.6%.

colour and drip loss. Multivariate modeling, however, improved the prediction of P_y.

3.4. Untargeted qualitative proteomic analysis by LC-MS/MS and protein database search

A PCA was performed with P_y as well as pH_u, L^* and drip loss as variables, which allowed the selection of 3 + 3 samples that represented opposite ends of the quality range based on the four variables (Fig. 5A). Importantly, if yellowness b^* and redness a^* were used in a PCA plot, then the positioning would be comparable with lightness (L^*). In addition, Fig. 5B illustrates that samples classified as poor (PQ) and good quality (GQ) based on PCA analysis also separate markedly with respect to P_y alone. Specifically, GQ samples ranged between P_y 55–73, and PQ samples between P_y 17–26.

GQ and PQ samples were then analysed to identify distinctive proteomic patterns that are linked to changes across the different quality attributes. LC-MS/MS analyses was performed on the sarcoplasmic fraction of the six selected muscle samples and revealed a total of 516 proteins. We identified 91 unique protein profiles for the group of PQ samples, and 164 unique proteins of GQ controls. 261 proteins were shared by both quality groups (Fig. 6). Importantly, this data shows that fewer unique proteins were detectable in samples that were classified as PQ samples through PCA.

Only proteins detected in all three samples belonging to one of the two quality classes are listed under poor (PQ) or good quality (GQ) in Table 2. Briefly, among the unique proteins we identified for the PQ samples were voltage-dependent L-type calcium channel subunit alpha, alpha-1-acid glycoprotein, heterogeneous nuclear ribonucleoprotein A2/B1 and apolipoprotein B mRNA editing enzyme catalytic subunit 2. For GQ samples we found junctophilin 1, cytochrome *c* oxidase subunit 5A, and adenylosuccinate synthetase isozyme 1. Unique GO terms were established by proteins from either “unique PQ “or “unique GQ”. GO terms revealed that “unique PQ” proteins were associated with biological processes, such as cytoskeleton organization, muscle contraction mediated processes and organelle organization (Supplementary Table 6). “Unique GQ” proteins were enriched in metabolism-associated GO terms, such as ATP metabolic process, generation of precursor metabolites and energy, as well as aerobic respiration (Supplementary table 4). In contrast to GO terms classified as “unique GQ” dataset, there were a fewer GO terms enriched in the “unique PQ” dataset.

4. Discussion

Here we explored the use of bioimpedance, a method previously used for assessing the structural integrity of biological tissue, to assess pork ham quality defects. Firstly, we established large variations in bioimpedance response among pork samples with varying degrees of meat quality defects, which were assessed with traditional quality monitoring approaches. Specifically, a reduced beta-dispersion, which is associated with compromised membrane integrity, was detected in a number of samples (Fig. 3A). We also found significant correlations between P_y and all tested traditional quality variables. Correlations were moderate for colour yellowness (b^*), redness (a^*), and drip loss, yet only weak for pH_u and lightness (L^*) (Fig. 4). Finally, we found that classifying ham samples based on BI responses together with pH_u, lightness and drip loss, allowed detecting unique protein profiles for good vs poor quality samples. Overall, our data indicates associations of bioimpedance response with standard quality variables as well as with proteomic differences. This supports bioimpedance to be an additional, useful marker in studies of pork defects, in particular if declining tissue integrity is to be monitored.

Our study corroborates a previous report that studied relationships between meat quality attributes and bioelectrical measurements (Pliquett et al., 2003). The moderate correlation, we detected between drip loss and P_y supports a link between the measured water exudate and

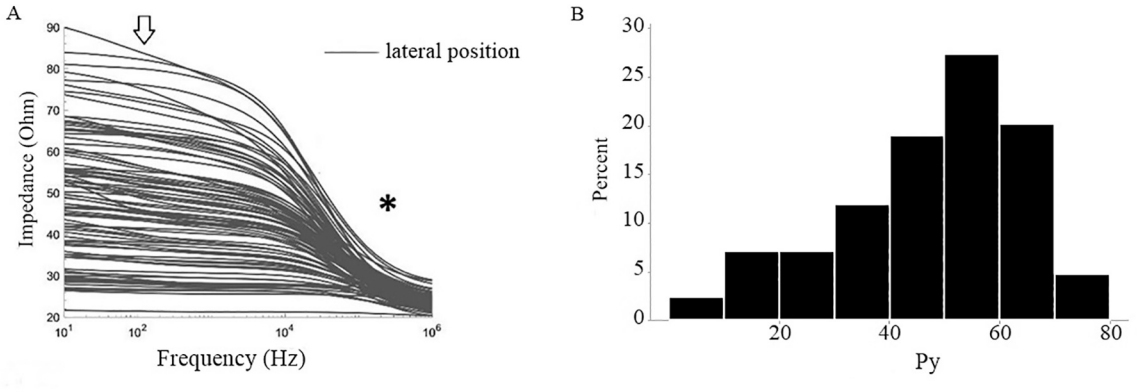

Fig. 3. Different bioimpedance responses for N = 84 samples indicated a large variation in tissue integrity for the same SM location as in Fig. 2. A. Typical for raw, fresh meat, all individual bioimpedance spectra showed marked alpha-dispersion (asterisk) and beta-dispersion (arrow). B. Also, P_y indicates a diversity in meat quality in the tested population, with P_y ranging from very low <6 to high <74 which provides information about cell membrane tissue integrity.

Fig. 4. Pearson correlation coefficients among the measured meat quality variables. Darker colours indicate stronger correlations between variables (orange = negative, blue colour = positive correlation). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

changes in muscle´ bioelectrical properties (Fig. 4). This means, that bioimpedance responses, may indeed help predicting drip loss data that is usually obtained 3 days later ($R = -0.31$, $P < 0.01$). Our data is in accordance with previous work that also confirms a pattern of moderate correlations between P_y testing and subsequent drip assessment after storage (Byrne, Troy, & Buckley, 2000; Pliquett et al., 2003).

Often colorimetric changes, including increased paleness, is interpreted as a result of changed light scattering due to protein degradation or due to changed myoglobin reduction state or concentration (Feldhusen, Warnatz, Erdmann, & Wenzel, 1995). In our study, correlations of bioelectrical measurements with colour differed for the three CIELAB colour coordinates (L^*, a^*, b^*, Fig. 4). We found the strongest correlations of P_y with redness (a^*) and yellowness (b^*). In agreement with our data, other authors present similar correlations between P_y vs. a^* and b^*, yet the practical use of this assertion varies depending on the goal of the study (Byrne et al., 2000; Najar-Villarreal et al., 2021). For surface lightness (L^*), however, such relationship remains uncorrelated suggesting that BI may be more sensitive to myoglobin concentration and its chemical state (a^*,b^*) – rather than scattering of the light (L^*) caused by protein changes due to decreased pH (Purslow et al., 2021). Importantly, the relationship of P_y with b^* may also suggest that cell membrane disintegration is accompanied by lipid oxidation. This is because the latter also affects the myoglobin chemical state, which is evident as a brown discolouration on the surface of affected meat (higher b^* value) (Chaijan, 2008).

In contrast to colorimetric measures and drip loss, we only detected a weak correlation between pH_u and P_y variables in our samples that were measured at 72 h post-mortem (Fig. 4). Previous studies on pork meat show that correlations between pH and P_y can be dynamic and decline in a time dependent manner (Byrne et al., 2000; Pliquett et al., 2003). Specifically, Pliquett shows a correlation between P_y and pH, when measured early post-mortem (pH_{45min}). However, such correlation became undetectable later (P_{y24h}). Moreover the P_y value may be influenced by meat type or mechanical stress (Altmann, Geisler, Schoeberlein, Pliquett, & Pliquett, 2000; Geissler, 1999). It is also conceivable that the pH/P_y relation may be different for different meat defects. For example, heritable PSE (Hal+) was a very common pork defect before 2005, i.e., the time the above studies were published. In (Hal+) pigs, a very sharp early post-mortem pH drop is thought to cause colour change, increased drip loss and tissue anomalies, and hence induce quality decline. This is despite pH values being comparably 'normal' when measured 24 h post-mortem – as compared to, e.g., RN- ('acid pork') defects, where very low pH values can be detected only later post-mortem and coincide with other quality anomalies (Salas Ramon Cesar & Mingala, 2017). In all, while our study confirms links among quality parameters, including P_y, correlations were typically moderate or weak, which is in line with other reports that explored such correlations (Antosik et al., 2022; Gjerlaug-Enger, Aass, Ødegård, & Vangen, 2010). This may suggest that individual test methods convey some unique information related to ham defects, and, therefore, can complement each other.

We found a significant effect of test location on P_y, and also on pH_u and the three colorimetric variables (Table 1, compare Fig. 1S). This underlines the need for proper identification and standardization of test locations for ham quality mapping, even if measurements are done on a single muscle. We here show that the lateral SM exhibits a lower pH_u and is generally paler (higher L^*) than the medial location. This suggests quality deterioration to be more manifest at lateral, typically thinner SM areas. It is, therefore, counterintuitive that we show higher P_y values at the lateral location. However, bioimpedance measurements can be confounded by a different set of factors than, e.g., pH and colorimetric tests. Importantly, our own observations point to a marked dimension effect of the tissue volume that the probe is inserted in, where different volumes exhibit different bioimpedance response. This again underlines the need to standardize test location, in particular if bioimpedance testing is to be used. Together, our data confirm links between standard

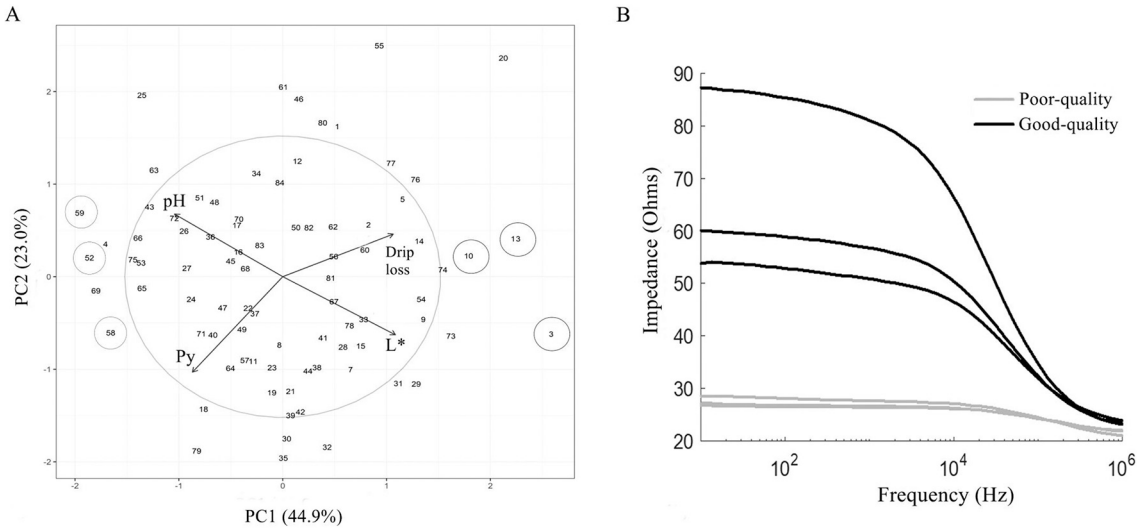

Fig. 5. Classification of good-quality and poor-quality ham samples. A. Selection was done based on PCA analysis including standard technological meat quality variables and bioimpedance measurement (pH_u, L^* (lightness), P_y and drip loss). Samples on the right represent extremes in poor quality (PQ) hams (low pH_u and Bioimpedance P_y, high drip loss and lightness). Samples on the left side represent extremes in good quality (GQ) hams (high pH_u and bioimpedance P_y, low drip loss and lightness). Selected samples for proteomic analysis are encircled B. Bioimpedance spectra for the selected samples, marked in fig. A. Two principal components were extracted explaining 67.9% of the variance of the initial data set.

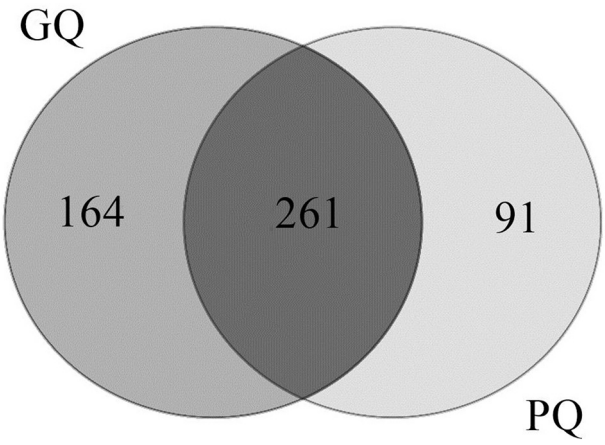

Fig. 6. Number of proteins identified in samples classified as good quality (GQ) versus poor quality (PQ). Unique proteins were found in either GQ or PQ hams, whereas shared proteins were found in both GQ and PQ pork hams.

quality features and P_y, which is to be expected for a multisymptomatic syndrome such as PSE-like pork. In addition, our correlation and PCA analyses also support that P_y carries some unique information, which is not expressed by standard quality parameters (compare Figs. 1 and 4).

Our proteomic analysis was performed to identify unique proteins for GQ and PQ samples, which were selected through PCA analysis based on 3 standard meat quality variables (pH_u, lightness, drip loss) and P_y (Fig. 5A). LC-MS/MS proteomic analysis resulted in 516 total extracted proteins, with 73 more proteins being detected in GQ samples (Supplementary Tables 1–3, compare Fig. 6). The overall lower protein count in PQ samples may be explained by an accelerated removal of proteins from the intracellular matrix through exudation in PQ samples. This is supported by higher drip loss and a lowered bioimpedance response, with the latter indicating translocation of water to extracellular compartments, which is facilitated by deteriorating membrane and fibrillar deterioration (Kleibel, Pfutzner, & Krause, 1983). Proteins removed from the intracellular space likely cannot be detected by using our proteomic fractionation protocol, as only sarcoplasmic proteins were targeted for extraction.

Table 2
Functional roles of representative differentially expressed proteins between the poor quality (PQ) and good quality (GQ) class meat, as identified by LC-MS/MS.

Protein	Quality class	Functional context	Gene Name/ Accession
Voltage-dependent L-type calcium channel subunit alpha	PQ	Mediates influx of calcium ions into the cytoplasm (Knox, Vinet, Fuentes, Morales, & Martínez, 2019)	CACNA1S/ A0A287B073
Alpha-1-acid glycoprotein	PQ	Anti-inflammatory responses (Fournier et al., 2000)	ORM1/F1SN68
Apolipoprotein B mRNA editing enzyme catalytic subunit 2	PQ	Enzyme protein (Sato et al., 2010)	APOBEC2/ A0A287BIN1
Heterogeneous nuclear ribonucleoprotein A2/B1	PQ	Enzyme protein and tumor biomarker (Liu et al., 2020)	HNRNPA2B1/ A0A286ZI52
Junctophilin 1	GQ	Tethering protein (Golini et al., 2011)	JPH1/F1RWK0
Cytochrome c oxidase subunit 5A	GQ	Energy metabolism	COX5A/F1SJ34
Adenylosuccinate synthetase isozyme 1	GQ	Energy metabolism	ADSSL1/ A0A287BAF3
Fumarate hydratase, mitochondrial	GQ	Energy metabolism	FH/ A0A287AR55
Lysine–tRNA ligase	GQ	Enzyme protein	KARS/ A0A287AK19
Transitional endoplasmic reticulum ATPase	GQ	Enzyme protein	VCP/ A0A286ZUM8
cAMP-dependent protein kinase inhibitor	GQ	Enzyme protein	PKIA/F2Z547

Apart from shared proteins found in both quality groups (Supplementary Table 6), proteins that were unique to either GQ or PQ groups often belonged to different GO terms, and hence, functional domains (Supplementary Tables 4–5). Specifically, GO terms linked to specific metabolic activities were enriched in the GQ samples, including ATP metabolic process, NADH metabolic process, and aerobic respiration process (Supplementary Table 4). This may suggest a prolonged activity for specific metabolic processes in this group until the respective energy

substrates are depleted (Bowker, Grant, Forrest, & Gerrard, 2000). In contrast to GO terms exclusive to GQ samples, for PQ samples the enriched GO terms included, e.g., cytoskeletal organization, organelle organization, and muscle contraction (Supplementary Table 5).

Among the specific proteins, unique to PQ samples (Table 2), was the Voltage-dependent L-type calcium channel subunit alpha (CACNA1S). This subunit is part of a membrane protein known as dihydropyridine receptor (DHPR), which is responsible for calcium signalling and controls the opening of the ryanodine receptor channel, a critical calcium gatekeeper, which is dysfunctional in pigs with hereditary PSE (Protasi, 2002). Studies done by (Wang et al., 2010) showed that also mutations in CACNA1S, i.e. upstream of the ryanodine receptor, can affect meat quality traits, such as ham weight or ham pH. Importantly, and resembling our findings, a recent study reported a higher abundance of CACNA1S in PSE meat (Liu et al., 2021). Plasma alpha-1-acid glycoprotein (ORM1) is another unique protein in PQ samples, which is an acute inflammation marker that was also implicated in tumor development (Fournier, Medjoubi-N, & Porquet, 2000; Matsusaka et al., 2021). Apolipoprotein B mRNA editing enzyme catalytic subunit 2 (APOBEC2), also specific for the PQ group, was previously implicated in striated muscle myopathies (Sato et al., 2010). Junctophilin (JPH1) is a protein unique to GQ samples and has a critical role in tethering, e.g., the ryanodine receptor-DHPR complex to cell membrane and endoplasmic reticulum. Thus, it stabilises the key elements responsible for proper trafficking of calcium ions from the extracellular space into the sarcoplasmic reticulum. In accord, JPH1 is known to be critical for calcium homeostasis (Golini et al., 2011). The above results support that multivariate selection of PQ and GQ samples allows identifying protein patterns that align with updated theories, where compromised function of the sarcoplasmic reticulum and subsequent impaired calcium homeostasis may play a role in emerging meat defects formation (Petracci et al., 2019). Given the potential significance of the proteomic data for understanding the role of specific proteins for the emergence of pork quality defects, comparable studies to test the reproducibility of our results are therefore warranted.

5. Conclusion

Bioimpedance measurements are sensitive to micro-structural damage and changes in water localization. Correlations exist with variables that are traditionally used for classification of pork meat. Our results also indicate that bioimpedance may provide additional information for detecting pork defects where tissue integrity within a meat cut is partly lost but not necessarily visible from outside. We therefore conclude that integration of bioimpedance testing in both, traditional quality monitoring protocols and in sample selection for omics investigations can inform about the structural integrity of meat products, i.e., beyond data that often solely relies on pH, drip loss or colorimetric testing.

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CRediT authorship contribution statement

Paweł Suliga: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Sisay Mebre Abie:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft. **Bjørg Egelandsdal:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Ole Alvseike:** Supervision, Writing – original draft, Writing – review & editing. **Amritha Johny:** Methodology, Software, Writing – review & editing. **Purushothaman Kathiresan:** Methodology, Visualization, Writing – review & editing. **Daniel Münch:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2022.108980.

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