Proteomics approach as a new way to predict tenderness as compared to classical South African Beef Carcass Classification System

by

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Objectives

- To use proteomics on beef samples to look for and quantify protein markers in muscle soon after slaughter that will predict tenderness potential of meat carcasses.
- To test suspected protein markers on unrelated samples to verify its efficacy as marker for carcass tenderness.

Justification

- Proteomics can be used to study the proteome after animal slaughter which can help the South African Beef Carcass Classification System (SABCCS) to accurately rate tenderness score on beef carcasses.

Introduction

- Currently controversy exists between feedlot beef producers and pasture beef producers which include poor farmers when rating the ultimate carcass tenderness.
- The South African Beef Carcass Classification System (SABCCS) does no accurately judge meat tenderness.
- It still discriminates against animal age at slaughter as an indication of potential tenderness.

- Various factors from the farm gate to the final cooked product affect not only tenderness but also other quality characteristics.
- Presenting a consistently high quality product is a combined effort by all role players in the industry to manage all the various quality factors (Troy & Kerry 2010).

- These factors include genetics, nutrition, growth promotants, pre-harvest stress, harvest technology (electrical stimulation, chilling), post-harvest conditions (duration of shelf life or aging, packaging, temperature) and cooking .
- At present it is still believed that tenderness is influenced by the age of the animal (Strydom *et al*, 2008 & 2011).
- This belief has been shown to be inconsistent and varies from one carcasses to another

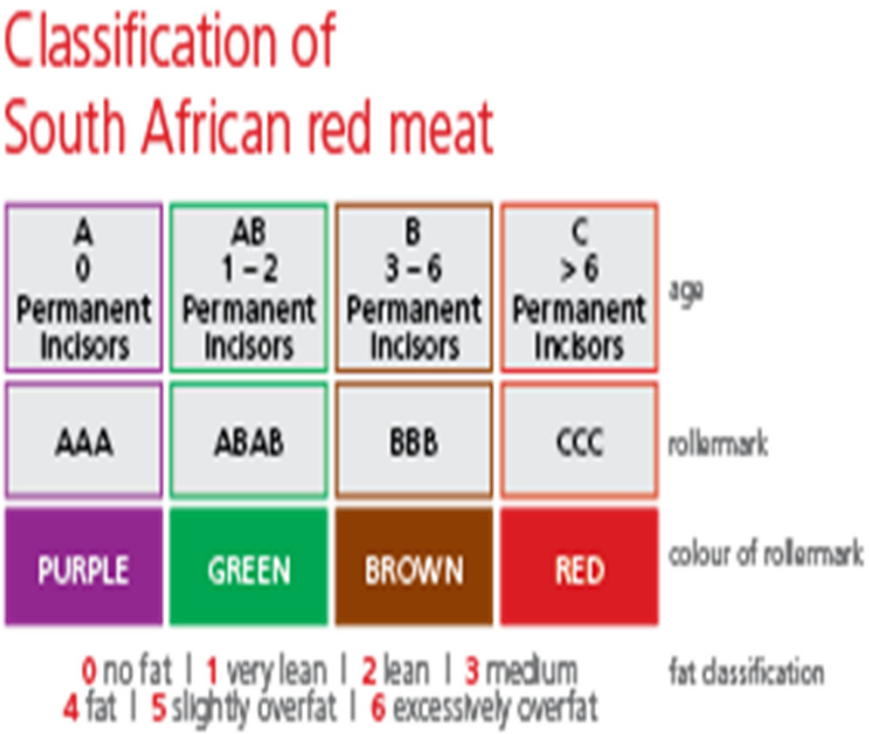


Fig 1: How the classification of red meat is conducted

The scientific basis of the classification is lacking, it only concentrate on the physical appearance which exclude the underlying biochemical activities. The question is should we rely on this physical appearances or we should start coming with the ideas of incorporating the biological ways to determine tenderness ?

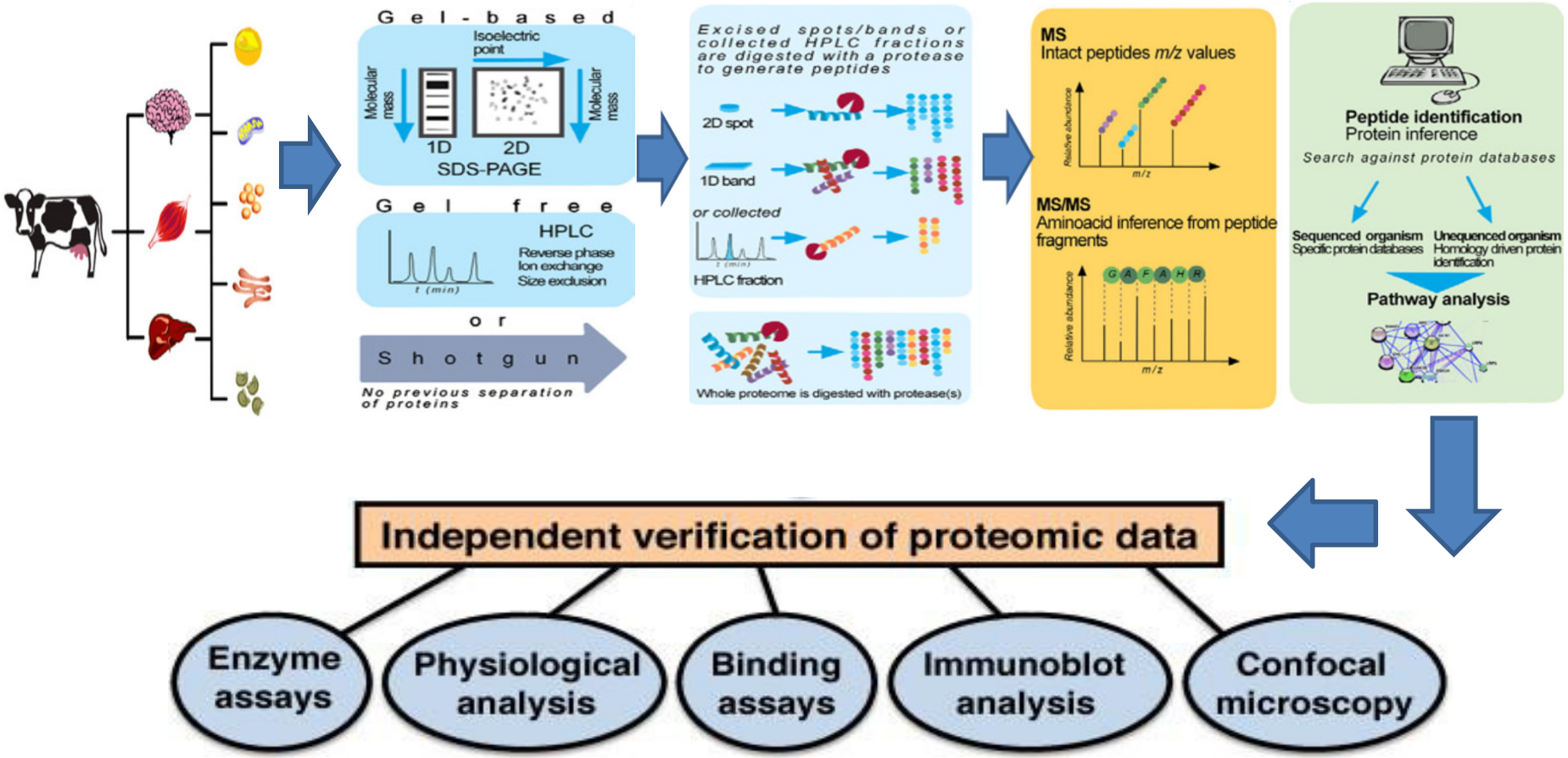
- Modern technologies such as the use of growth stimulants and electrical stimulation influence tenderness.
- Therefore the SABCCS should be adapted to take these technologies into account.
- Two-dimensional gel electrophoresis (2-DE) is a useful technique which has a promising potential to identify differentially expressed proteins that might be associated with meat quality (Tambor *et al.*,2010) .

- Such differentially expressed proteins could be implemented as molecular biomarkers for meat quality and may provide new insights into the molecular mechanisms and pathways related to marbling and tenderness (Jia *et al.*,2006, Lonergan *et al.*, 2010).

Methodology

- The following Breeds will be studied –Brahman, Nguni, Angus, Charolais and Bonsmara, 10 animals from each breed will be studied.
- Muscle samples will be taken between the 11^{th} and 12^{th} rib, 1 and 24 hours post-slaughter from each animal.
- Other samples will be taken 3, 9, 14 and 21 days post-slaughter.
- These samples will be frozen with liquid nitrogen and stored at -80 °C until processing.

Schematic representation of proteomic workflow (bottom up)



- Two-dimensional gel electrophoresis (2D-SDS-PAGE) of individual samples per breed will be performed.

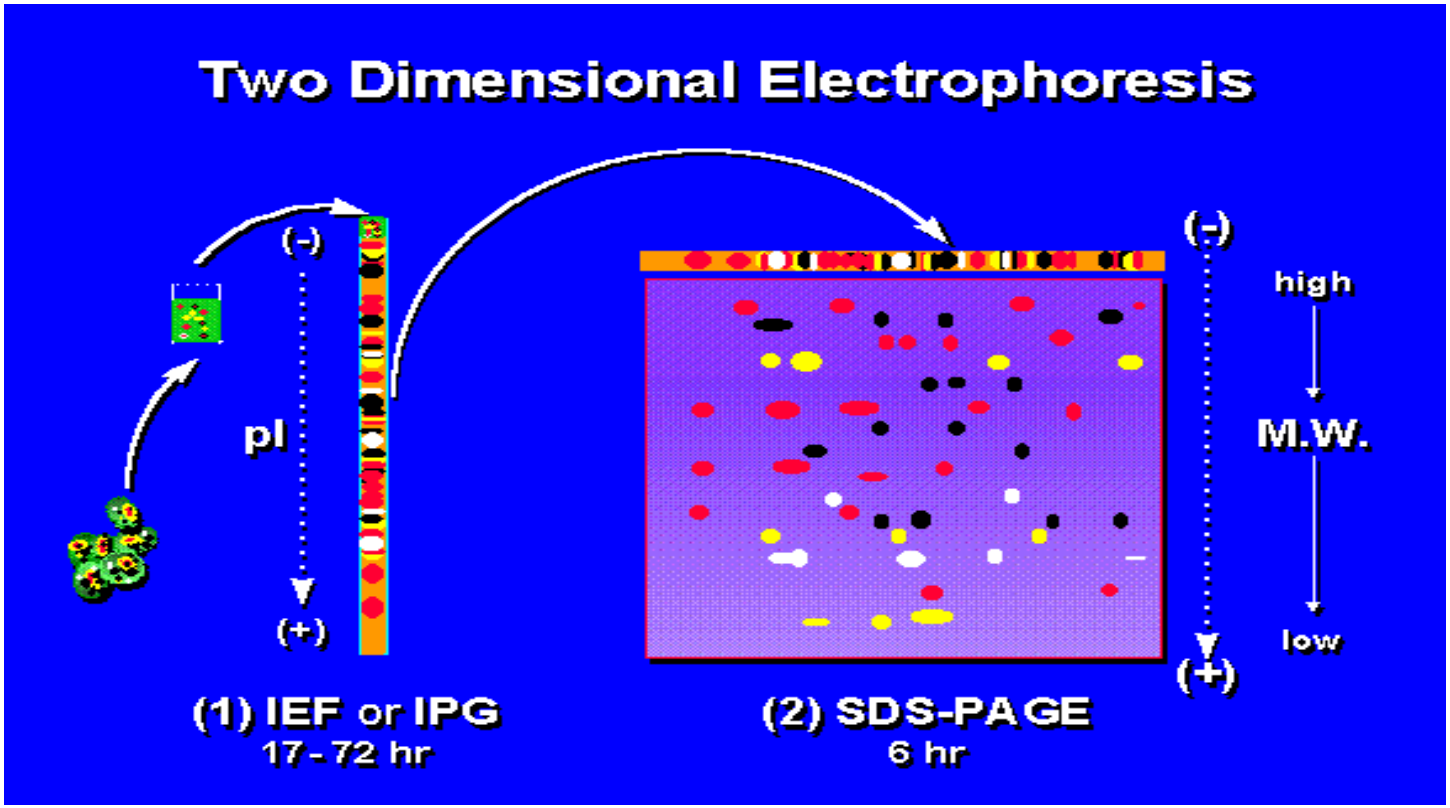
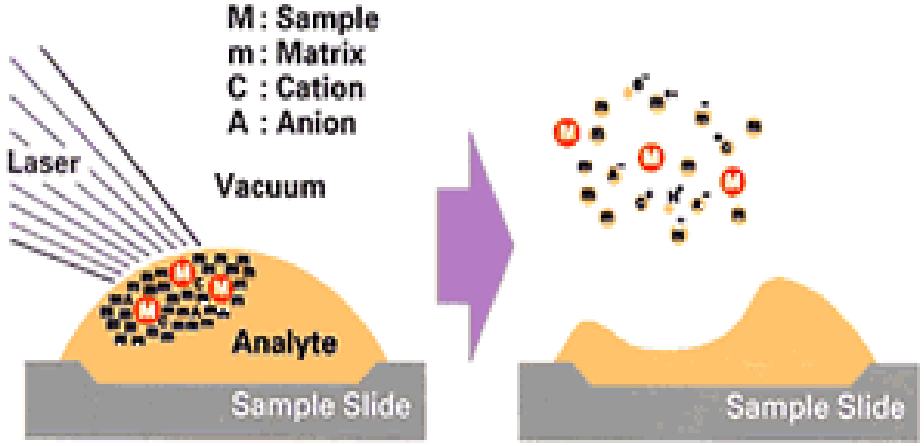


Fig 2: Summery of how the 2D SDS PAGE will be carried out.

- The protein map will be analysed by PD Quest software from Bio-Rad to evaluate the qualitative and quantitative difference that might be arise from breed samples and aged samples between 3, 9, 14 and 21 day post slaughter,

- Proteins of interest will be identified and quantified with matrix-assisted laser desorption ionization (MALDI) time of flight (TOF/TOF) mass spectrometry.



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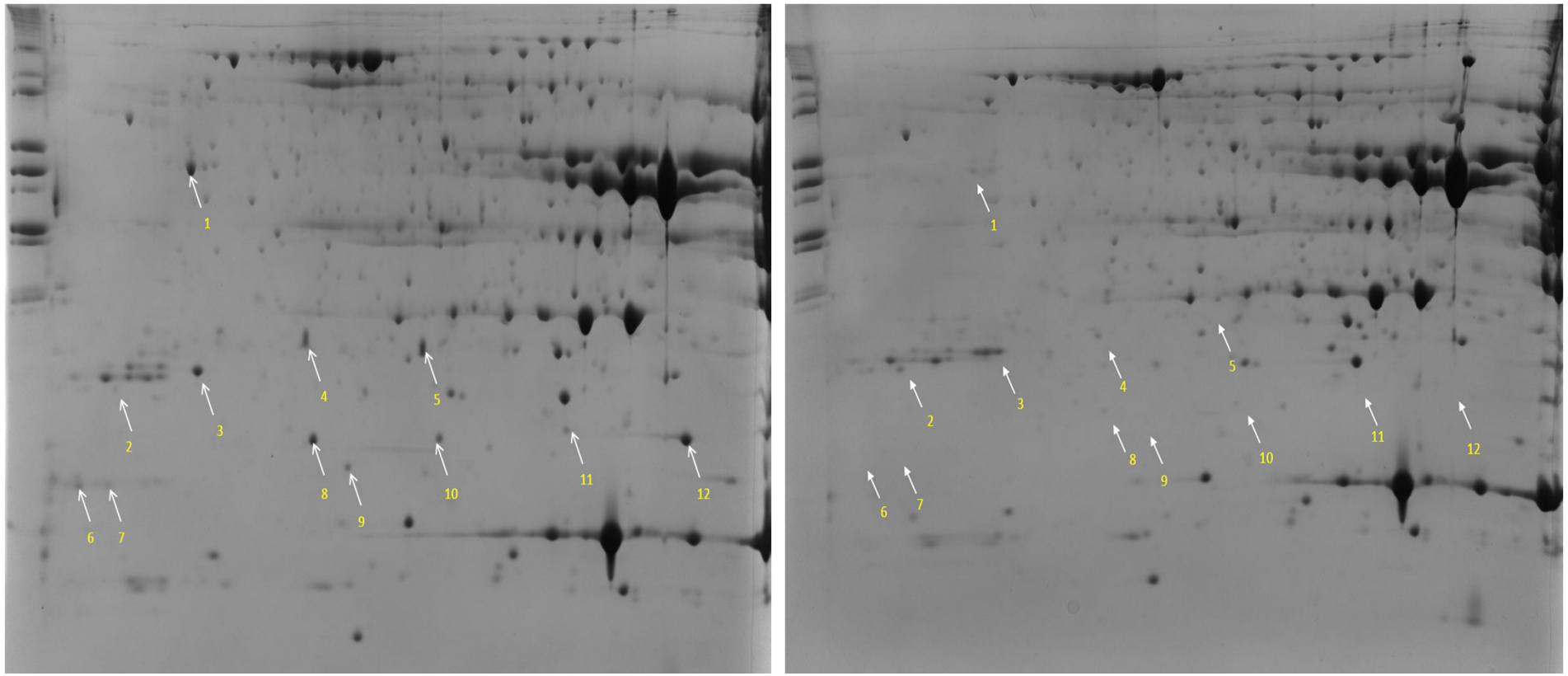
- The identified proteins will be confirmed by western blot or ELIZA.
- Enzymes such as calpains will be also determined according to standardized methods at Biochemistry, Meat Science – ARC-API.

Results



A Day 0

B Day 3

Figure 1. Gels from Nguni sample showing differences in protein expression from day 0 and day three. Gel A shows proteins present in day zero and gel B shows proteins absent in day zero in day three.

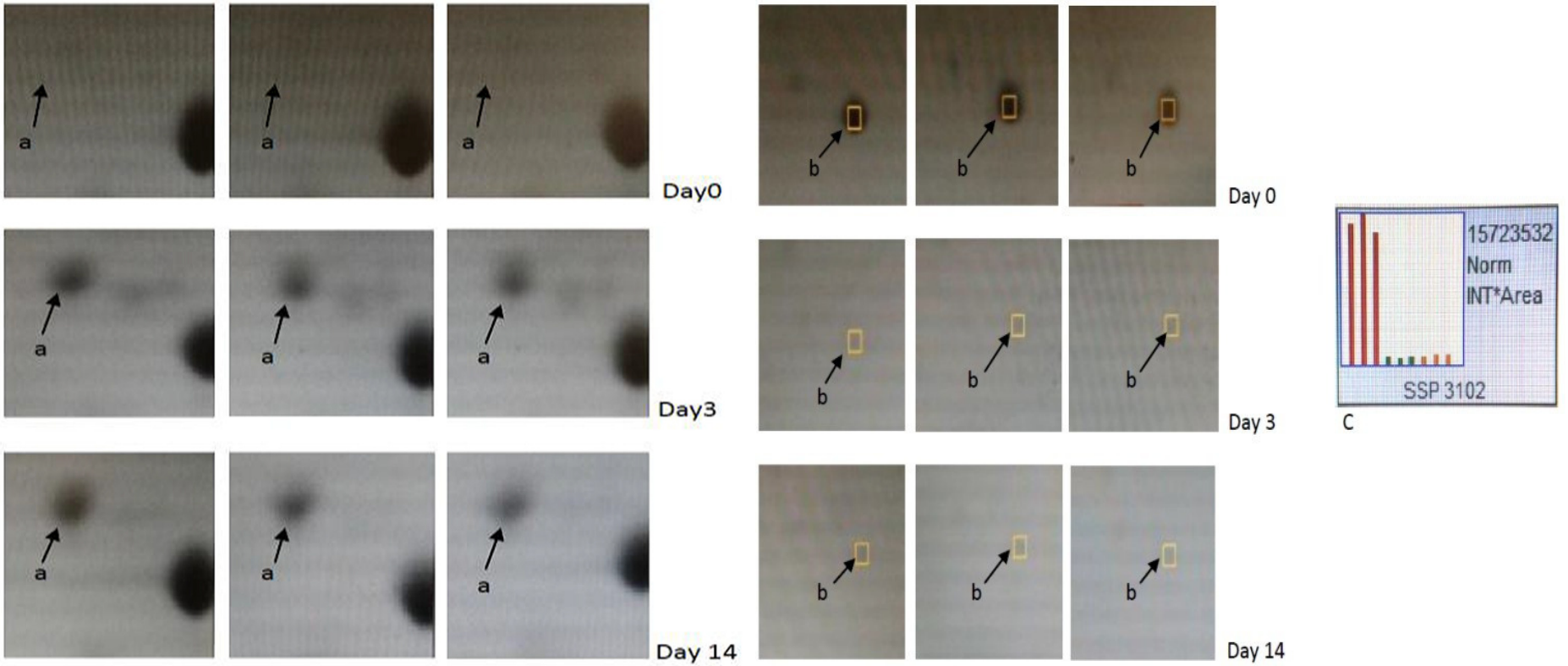


Figure 2. A zoomed in gel section of representative spots showing differential expression patterns after several days of ageing at 0-4 °C. IEF pH range is 5–7, 12% T 3% C Acrylamide. Gels have been stained with Coomassie Brilliant blue G250. The expression of the spots in B are also shown in PDQuest software bar graphs to illustrate the effect of ageing.

Table 1. Average Warner Bratzler shear force (WBS) and myofibril fragment length (MFL) measured in animal N50 *M. longissimus lumborum* showing decreases as ageing progress.

Characteristics	N50 Day 3	N50 Day 14
WBS (kg)	6.37	4.67
MFL (µm)	35.73	22.53

Conclusion

- In this study several proteins have shown a change in response to ageing such as proteins marked in figure 1 A and B and proteins a and b in Figure 2. At this time we don't know if their change is caused by ageing or other factors. In the next phase of the study, these proteins will be identified with mass spectrometry.

Acknowledgement

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