Search for protein markers related to beef tenderness in an indigenous South African breed using a proteomics approach.

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ABSTRACT - The current South African Beef Carcass Classification System uses age as only indicator of tenderness implying that optimal tender meat is achieved from carcasses from animals with no permanent teeth. Research show that if considering modern technologies (use of beta-agonists, growth hormones and electrical stimulation amongst others), this is not necessarily true. We are searching for protein markers that are related to tenderness to use as tenderness predictors. Twenty IRENE feedlot Nguni animals with 0-teeth were slaughtered. Samples were collected from *M. longissimus lumborum,* snap frozen and stored at -80 °C until analyses. Soluble proteins were extracted using TES buffer followed by two-dimensional-gel-electrophoresis. Results show that several proteins change in response to ageing but we are focusing on two examples; protein spot a which is absent at 1h post-mortem but as ageing progress the spots start to appear as seen in 3 and 14 d post-mortem and protein spot b showing high expression level of at 1 h post-mortem but little at 3 and 14 d post-mortem. These differential expressions corresponded with shear force and myofragment length measured at 3 and 14 d post-mortem, sugesting that ageing because of proteolytic action (calpains and calpastatin system) could have caused the change.

Keywords: Protein markers for tenderness; Nguni breed; Two dimensional gel electrophoresis (2-DE).

I INTRODUCTION

Where meat is concerned and especially beef most consumers worldwide consider tenderness as the most superior quality attribute compared to juiciness and flavour (1). Because tenderness is regarded as the main quality attribute, this puts quality assurance systems under pressure to guarantee consistently tender meat. Tender meat is the sum result of all role players in the meat industry to manage various factors including genetics, nutrition, growth promotants, pre-harvest stress, harvest technology (electrical stimulation, chilling), post-harvest conditions (duration of shelf life or ageing, packaging, temperature) and cooking (2). Currently controversy exists between feedlot beef producers and pasture beef producers which includes poor farmers because the South African Beef Carcass Classification System (SABCCS) does not accurately judge meat tenderness, but still discriminates against animal age at slaughter as an indication of potential tenderness (3). Modern technologies such as the use of growth stimulants and electrical stimulation influence tenderness and therefore the SABCCS should be adapted to take these technologies into account. There is dire need of technology that can be used to classify the beef carcass based on its true tenderness and eliminate the biasness of age alone. One of the potential and promising technologies is proteomics. Proteomics can be defined as a fast developing field of biochemistry. Proteomics are a wide scale study of the complete proteome in an organism, tissue or cell and the mechanism of interactions between the expressed proteins (4). Proteomics as compared to its sister study genomics is far more complex due to various transformations that proteins undergo after translation, involving protein isoforms, post-translation modification (e.g. glycosylation, phosphorylation), and protein-protein interactions. Proteomic structure as compared to genomics is easily altered or affected by a wide variety of internal and external factors such as environment, age, sex, diseases, and nutrition. Since the inception of the term proteome in 1994, there were various analytical methods evolved or developed to study and understand in depth how proteomics functions. Out of different method developed, two analytical

methods stand out to be more and widely used i.e. two-dimensional-isofocusing–sodium-dodecyl-sulphate-poly-acrylamide-gel-electrophoresis (2-DE) which separates proteins based on their pI and molecular weight followed by identification of proteins by mass spectrometry (MS). These technologies have promising power to identify proteins that can be used as bio-markers for beef carcasses for tenderness.

In this study we used Nguni animals to verify our search for protein markers on our way to identify potential tender carcasses using 2-DE and then MS. From previous studies the Southern African indigenous Nguni breed showed genetic potential to produce tender meat (5.6) when using correct pre- and post-slaughter procedures. This breed is an example of being discriminated against by the SABCCS because Nguni is not well adapted to feedlot systems and is rather used in extensive farming systems and has 2 to 4 permanent teeth by the time they are market ready.

II MATERIAL AND METHODS

Animals and sampling

The indigenous Southern African Sanga breed (Nguni) was used in this preliminary study. Twenty animals which were fed normal feedlot diet were slaughtered at the IRENE abattoir. Samples were collected in different days post-slaughter (D0 within 1 h post-slaughter, D3 and D14 post-slaughter) snap frozen with liquid nitrogen and stored at -80 °C until further analysis.

Extraction of muscle proteins

The frozen muscle (200 mg) was homogenised in 1 ml TES buffer and extracted according to Jia *et al*. (7). Protein concentrations were measured with a commercial kit at 750 nm (RC-DC Protein Assay, Bio-Rad) in an ELX Universal micro-plate reader with BSA as a standard. Individually extracted samples were analysed by means of 2-DE. Each sample was run in triplicate to evaluate the technical variability.

Two-dimensional gel electrophoresis

Protein separation in the first dimension was performed on an immobilised pH gradient (IPG) strips (Bio-Rad), 24 cm, spanning the pH region 5-7. Proteins (700 µg) were loaded onto each IPG strip by in-gel rehydration overnight at room temperature. Isoelectric focusing was performed using the Ettan IPGPhor II unit (GE Healthcare BioSciences, Uppsala, Sweden) using a stepwise programme described as follows; 500 V for 2h, increase to 1000V for 2h, increase to 10000 V for 3h, 10000V for 7:36h (seven hour thirty six minutes). The second dimension proteins were separated on 12% SDS-PAGE using the Ettan DALT six large format vertical system (GE Healthcare Bio-Sciences). Gels were stained with Coomassie brilliant blue G250.

Image acquisition and analysis

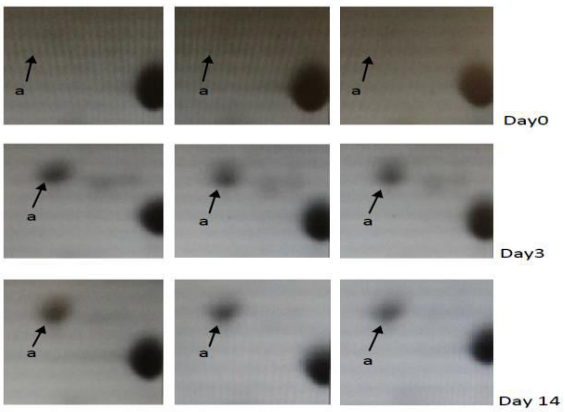
Gels were imaged using Chemi-doc Mp (Bio-Rad Hercules, CA, USA) equipped with Image Lab software. Comparative analysis of the expressed proteins of two samples within the breed was carried out using PDQuest Advanced 2D analysis software. Each sample had three gel replicates. Gels were normalised using the group consensus tool. Differentially expressed protein spots were statistically significant using the student t-test at 95% significant level.

Tenderness was measured mechanically by means of Warner Bratzler shear force (WBS) (8).

The myofibril fragments (MFL) from filtrate from extracts from LL were examined with an Olympus BX40 system microscope and a 400X magnification. 100 Myofibril fragments of each sample were measured, using a software package (analySIS Life Science) (9).

III RESULTS

These results shown here are part of the study of searching for protein bio-markers related to tenderness especially in the South African beef breeds. Two-dimensional electrophoretic analyses were (was) performed in biological triplicates on protein extracts from *M. longissimus lumborum* samples. Triplicates allowed increasing significance of the statistics behind spots showing differential photo densities.



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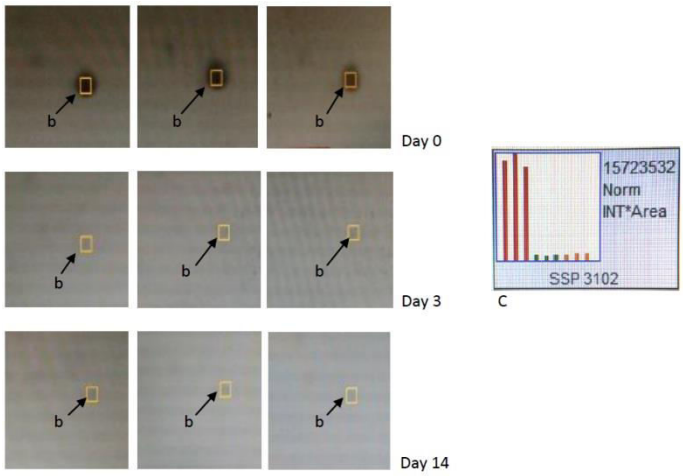

B

Fig. 1 A zoomed gel section of representative spots showing differential expression patterns after several days of ageing between 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. WBS and MFL decreases as ageing progress.

Characteristics	**N50 D3**	**N50 D14**
WBS (kg)	6.37	4.67
MFL (µm)	35.73	22.53

The protein spots indicated in Figure 1 were statistically significant using student's t-test at 95% significance level. Figure 1 A shows a protein spot which is absent in D0 but as ageing progress the spot start to appear as seen in D3 and D14. For this study the molecular range of the proteins were not determined but the pH range was located between 5-7. The graphic and computer based image analysis of the 2D gels help in pinpointing the differentially expressed proteins as ageing progress. The quantity/expression of the spot a in D3 and D14 are almost similar suggesting that ageing from D3 to D14 did not have much effect on spot a. Spot b in Figure 1B shows high expression level in D0 but little in D3 and D14. The expression of spot b is similar to spot a in the same Figure 1 A with the difference that spot a is not present in D0. The expression of spot b is also shown on the PDQuest generated bar graph in Figure 1C. As can be seen on the bar graph the expression levels of spot b is highly expressed in D0. The spots expression seems to relate with WBS and MFL related to tenderness shown in Table 1. The WBS decrease with ageing with the value of 6.37 kg at D3 and 4.67 kg at D14. The same pattern is followed by the myofibril fragment length (MFL) which is 35.37 µm at D3 and 22.53 µm at D14. The names and properties of these proteins are yet to be determined by mass spectrometry (MS).

IV DISCUSSION

In this introductory investigation we evaluated the protein patterns in relation to ageing to study their post mortem behavior. The expression patterns of 2D gels in most cases reveal more information than the naked eye can recognise. With the use of PDQuest, differential expression was made possible. In this study several proteins (43 spots) have shown differential expession in relation to ageing. For example in Figure 1A spot a is not detected in D0 but fully detected during the ageing progress. Also in Figure 1B spot b is highly expressed in D0 but expression decreases as ageing progress. There are several factors that may be responsible for these changes in differencial expresssion such as ageing because of proteolytic action (calpains and calpastatin system and or cathepsins) (10). Intermediate pH affects the calpains and cathepsins negatively as they are less optimal in that pH range. After slaughter there are a lot of changes taking place in the carcass when the muscle changes to meat. As muscle goes into rigor, there is a loss of extensibility and along with that, a change in the texture of the meat (11). There are more than a few

key proteins that are modified during post mortem ageing such as actin and myosin. Those proteins are located in different regions of the muscle cell, and most have been implicated in some way as being vital in maintaining the structure and function of the muscle cell. There were several studies that focused on probing this proteins as biomarkers for tenderness. Searching for biomarkers related to tenderness has presently gained much interest in the field of meat science. A biomarker is a characteristic that is objectively measured and assessed as a pointer of normal biologic responses to interference (12). It has to be able to fulfill certain criterion such as unbiased diagnosis. It offers a protein, the presence or quantitative characteristics of which are measured.

V. CONCLUSION

In this study several proteins have shown a change in response to ageing such as proteins a and b in Figure 1. 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.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the following committees of institutions: The Agricultural Research Council (ARC) for facilities and financial support. The Red Meat Research and Development of South Africa and the Technology and Human Resources for Industry Programme of the Department of Trade and Industry, South Africa for the funding of this project.

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