Proteins related to tenderness in the South African indigenous Nguni breed

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Abstract - By identifying the suitable tenderness markers proper practice can be developed to control the process profitably and to guarantee meat tenderness. In this study proteins were extracted from frozen meat sampled from M. longissimus lumborum (LL). Proteins were separated by means of immobilised pH gradient (IPG) strips using Ettan IPGPhor II unit and SDS-PAGE. Gels were stained with coomassie brilliant blue (G250) and imaged by Chemi Doc Mp imaging system. The results in this study show that several metabolic, structural and stress proteins changes with ageing. Some of the metabolic enzymes such as adenylate kinase are involved in the energy production in the electron transport chain while other are involved in glycolysis and citric acid cycle. The stress induced phosphoprotein, heat shock proteins and Polyubiquitin-C were also found to change in abundance in LL as ageing progresses. The pattern of expression with tenderness is yet to be determined. The results suggest that during ageing the metabolic, stress and structural proteins are affected. In this study proteins showing a significant change in relation to ageing were identified and some could be possible tenderness markers. The proteins identified from Nguni breed are not different from other proteins identified from different breed worldwide.

Key words Biomarkers, 2D-SDS-PAGE, metabolic and stress proteins, ageing.

I INTRODUCTION

Several proteomic studies have revealed that many of the detected post mortem proteome changes are associated with protein degradation progressions. Proteomics studies has a possibility of providing the discovery of biomarkers that can be of important indication to tenderness and help to decrease meat quality inconsistency and also facilitate management decisions. Biomarker marker refers to a quantifiable indicator of certain biological circumstance, such as tenderisation. Consumers are prepared to pay extra for superior quality meat products with guaranteed tenderness [1][2][3]. Meat tenderness can be affected by various factors such as age, environment, stress during pre-slaughter, post-slaughter handling, the rate of glycolysis, sarcomere shortening during rigor, the ultimate pH, and the extent of myofibrillar protein degradation during ageing or storage, the amount of connective tissue and other unknown underlying metabolic factors. The effect of stress during pre-slaughter is well described in the review of Ferguson & Warner [4]. According to Kuchel [5] the secretion of catecholamines in stressed animal have a substantial change in energy metabolism, lipolysis, and glycogenolysis in muscle as well as gluconeogenesis. These may affect meat tenderness negatively as shown in a study by Chulayo & Muchenje [6]. The activity and expression levels of glycolytic enzyme systems including cathepsins, the calpain/calpastatin system and the proteasome can also affect meat tenderness, see the review by Bendixen [7]. In this study the two dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (2D-SDS-PAGE) was used to investigate the changes in protein level or patterns related to meat tenderness in South African indigenous Nguni breed.

II MATERIALS AND METHODS

Meat samples were collected from M longissimus lumborum (LL) of Nguni beef carcasses acquired from ARC-API abattoir Irene South Africa, after animals were raised from ARC-API feedlot Irene South Africa. The animals received a normal feedlot diet. Meat samples were sampled one hour after slaughter and snap-frozen by liquid nitrogen and stored at -80 °C. Other samples were aged for three and fourteen days, after ageing they were also frozen by liquid nitrogen. The frozen muscle (200 mg) was homogenised in 1

ml TES buffer as formulated according to (Jia et al [8]. The Precelly homogeniser (Bertin, le Bretonneux, France) was used at 5500 rpm, 2 x 20 sec; 10 sec pause. After homogenising, the contents were centrifuged (30 min at 13000 rpm at 4 °C) to remove TES insoluble proteins. Protein concentrations were measured with a commercial kit at 750 nm (RC-DC Protein Assay, Bio-Rad, USA) based on Lowry assay in an ELX Universal micro-plate reader at 750 nm with BSA as a standard. Protein separation in the first dimension was performed on an immobilised pH gradient (IPG) strips (Bio-Rad, USA), 24 cm, pH 5-8. Protein extracts containing 850 µg were loaded onto each IPG strip by passive rehydration for overnight at room temperature. Isoelectric focusing (IEF) was performed using the Ettan IPGPhor II unit (GE Healthcare BioSciences, Uppsala, Sweden) by means of a stepwise programme described as follows: 500 V for 2 h, increase to 1000 V for 2 h, increase to 10000 V for 3 h, 10000 V for 7:36 h. 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 by coomassie brilliant blue (G250) prepared as follows: 100 g of ammonium sulphate was dissolved into 650 ml of double distilled water, a 1 g of coomassie G250 was added and stirred until coomassie had dissolved. A 30 ml of orthophosphoric acid was added to the mixture followed by 200 ml of 99% ethanol and followed by double distilled water to make up 1000 ml. Gels were fixed for 60 min in fixing solution (o-phosphoric acid 85% 1.3% w/v and methanol 20% v/v) and stained for overnight. After staining, gels were transferred in neutralisation buffer (Tris-base 0.1 M pH 6.5 by o-phosphoric acid) for 1-3 min, and washed with 25% methanol for 1 min and stored in stabilising solution (20% ammonium sulphate). Gels were imaged and processed using Chemi-doc Mp (Bio-Rad Hercules, CA, USA) equipped with Image Lab software. Proteins were identified by Maldi Tof-Tof spectrometer (Information).

II RESULTS AND DISCUSSION

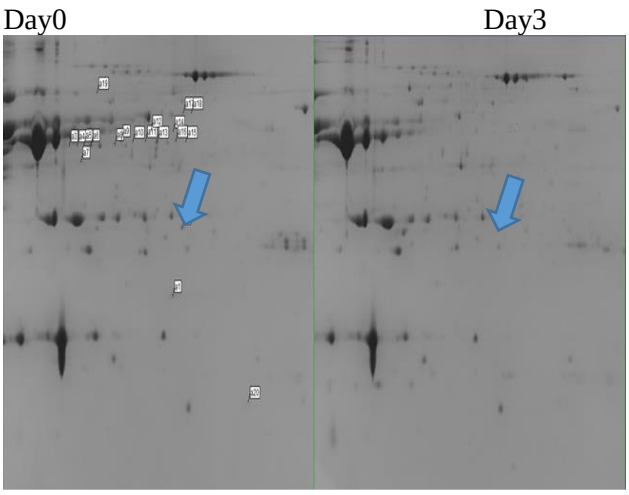
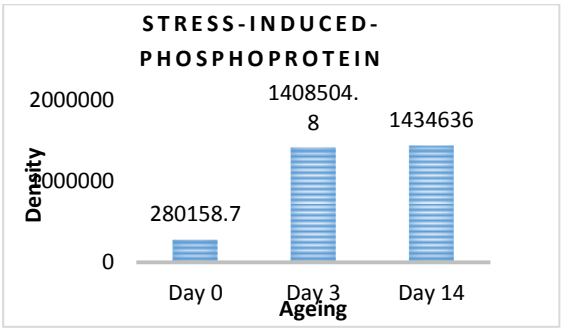
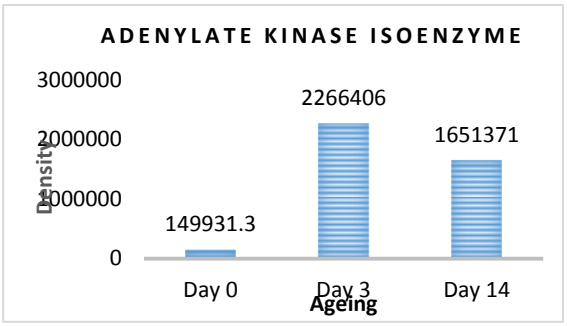
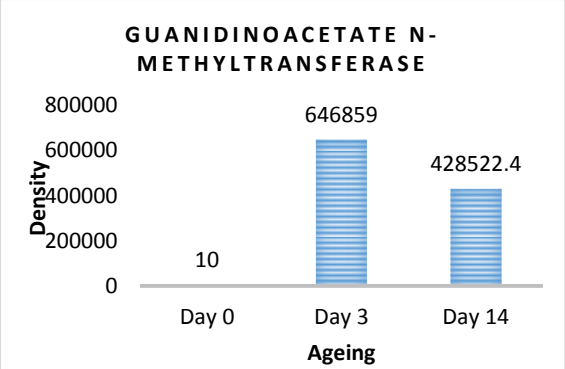


Figure 1. Gel images from LL extracts of Nguni breed showing differences in protein expression between ageing day 0 and day 3 post mortem. The annotated protein spots are not expressed in day 3.







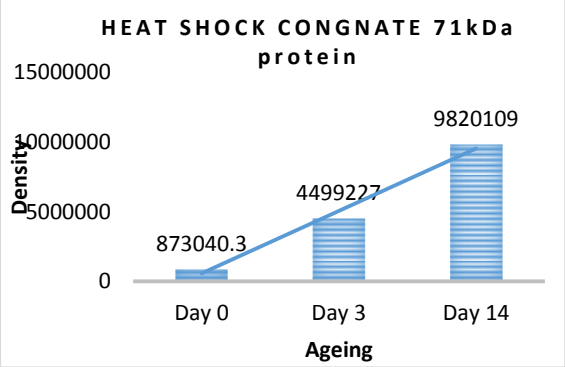


Figure 2. The density (levels) of the spots expression of stress induced phosphoprotein, adenylate kinase isoenzyme and heat shock cognate 71 kDA protein of LL from Nguni breed

Table 1 List of the identified proteins spots showing difference in expression in relation to ageing of Nguni LL.

Proteins spot identified
Heat shock cognate 71 kDa protein
Troponin C,
Troponin T
Malate dehydrogenase
Adenylate kinase isoenzyme
Aldose reductase
Myosin light chain
ATP synthase
Tropomyosin beta chain
Adenylate kinase isoenzyme
Creatine kinase
Haloacid dehalogenase
Polyubiquitin-C

The results in this study were analysed using the PD quest software (Bio-Rad Hercules, CA, USA) with build in statistical software. The student t-test was used to explore the differences in variation in the comparative 2D gels patterns. The comparative analysis of the expressed proteins between three ageing periods being day 0, 3 and 14 were carried out. In Figure 1 the results show the abundance of NADH dehydrogenase (indicated by arrows) which is located in the mitochondrial inner membrane and is amongst the five complexes of the electron transport chain. It is expressed in the early post mortem at day 0 as part of energy production to keep muscle cell active. As ageing progresses, it gets depleted as shown in day 3 of ageing. On the other hand, the metabolic adenylate kinase isoenzyme increases in abundance, showing how the cell is striving to provide energy to the muscle cells. This enzyme catalyses the reversible reaction of adenine nucleotides, and performs a significant role in cellular energy homeostasis. The results are consistent with the results found by Jia et al. (2007) whereby metabolic enzymes adenylate kinase, GPD1 protein and Guanidinoacetate N-methyltransferase increased early post mortem. These abundant changes in metabolic enzymes shows that the cell has to develop survival mechanisms to prevent cell death. The improvement of cell capability to provide the energy needed for increased metabolic activities is also vital. During aerobic conditions glucose is converted to pyruvate subsequently converted to acetyl Co-A, but in anaerobic conditions pyruvate is converted to lactate resulting with only two adenosine triphosphate (ATP) molecules instead of 34 ATPs for each glucose molecule used up during aerobic conditions. At present the study shows the number of stress proteins such as stress induced phosphoprotein, heat shock proteins and Polyubiquitin-C changes in abundance in LL as ageing progresses. Heat shock cognate 71 kDa protein enables the appropriate folding of newly translated and misfolded proteins, in addition it is involved in the degradation of mutant proteins. It also plays a role in biological processes such as signal transduction, apoptosis, protein homeostasis and cell growth. The expression of these proteins show how the cell is attempting to preserve the normal functioning of the cell under stressful conditions. The expression of these stress protein in relation to ageing might be used to correlate them with tenderness. The pattern of expression with tenderness is yet to be determined. The results suggest that during ageing the metabolic, stress and structural proteins are affected as shown in Table 1. Other structural proteins such as the troponin C, troponin T, myosin light chain, tropomyosin beta chain were also shown to change in abundance in relation to ageing. The structural changes show the destabilisation of structural integrity of the

sarcomere. The troponin complex is a significant regulator of actin and myosin cross linking in skeletal muscle. Degradation or any other disruption to these proteins have the possibility of affecting the overall tenderness of the muscle over the weakening of the myofibrillar structure. The overall results are consistent with the results extensively reviewed by Ouali et al. [9] whereby several proteins ranging from metabolic, stress induced proteins, structural proteins, proteasome and miscellaneous proteins were found to be changing with meat ageing making them possible tenderness markers.

IV Conclusion

The results show that several metabolic, structural and stress proteins changes with ageing. The expression of metabolic related proteins during early post mortem suggest that the cell is striving to produce energy even under stressful conditions, this is supported by expression of stress proteins which may delay cell death for a while. Though the understanding of factors such as temperature, pH, electrical stimulation water holding capacity affecting meat tenderness are understood and well-studied including the possible proteins which can be used as markers for tenderness, there is high short fall when coming to the mechanism of interplay of those factors and the possible proteins. From this study proteins showing a significant change in relation to ageing were identified and some could be possible tenderness markers. The proteins identified from Nguni breed are not different from other proteins identified from other breeds worldwide although the extent of expression might differ. This results shows that a possible universal protein marker for tenderness is possible. Although more intense studies are required in order to establish protein markers for tenderness.

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