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Changes in pH, colour and the microbiology of black wildebeest (*Connochaetes gnou*) *longissimus thoracis et lumborum* (LTL) muscle with normal and high (DFD) muscle pH

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ABSTRACT

The effect of pH and the spoilage of black wildebeest *Longissimus thoracis et lumborum* (LTL) muscles with normal (pH > 6.06) and high pH (DFD; pH < 6.06) was investigated for 12 days under refrigerated (5 ± 1 °C) aerobic conditions. Results showed that pH affected colour, as initial values from Normal samples (L* = 33.08, a* = 13.60, b* = 10.29, C* = 17.10 and H_{ab} = 36.85) were greater than values for DFD meat (L* = 27.21, a* = 11.10, b* = 6.97, C* = 13.12 and H_{ab} = 32.08). Initial bacterial counts from DFD and Normal pH samples did not differ significantly. Over time, pH decreased for Normal and DFD samples until the 6th and 9th day, respectively, whilst both samples showed a significant decrease in redness and colour intensity. Aerobic bacteria and *Enterobacteriaceae* reached 7 log cfu/g > 4 days earlier than Normal pH samples and bacterial growth rate was > 1.09-fold faster in DFD than Normal meat.

1. Introduction

Prolonged stress or high activity before slaughter can result in the depletion of glycogen stores in the muscle. This can cause a limitation of post-mortem glycolysis which can result in low lactic acid production and maintenance of a higher pH than normal in the muscle/meat of an animal post-mortem (Kritzinger, Hoffman, & Ferreira, 2003). As a consequence, high ultimate muscle pH can lead to the formation of dark, firm and dry (DFD) beef, mutton and to a lesser extent, pork meat (Newton & Gill, 1981). Additionally, DFD game meat from South Africa has been reported to occur due to the highly stressful manner in which game animals are hunted (Hoffman, 2000). Ultimately, muscle pH will change the quality and safety of meat, as DFD meat has been reported to have a short shelf-life and a poor appearance (Wiklund & Smulders, 2011).

The colour of meat is one of the most important indicators of meat quality and plays an important role in consumer's purchasing decision in buying the product or not (Mancini & Hunt, 2005; Neethling, Suman, Sigge, Hoffman, & Hunt, 2017). Colour can indicate flavour, tenderness, safety and freshness to consumers (Hoffman, Muller, Schutte, Calitz, & Crafford, 2005). DFD meat allows for further deterrent to consumers, as the meat appears to be even darker (Swatland, 1990). In terms of discolouration, it is important to note that even though consumers use colour as an indicator of freshness; discoloration in meat actually can occur prior to microbiological spoilage (Young & West, 2001). South African game meat has been perceived to have a dark unappealing colour to consumers, mainly due to the high myoglobin content (Hoffman, Mostert, Kidd, & Laubscher, 2009; Wiklund & Smulders, 2011).

Previous researchers have accredited the shortened shelf life of DFD meat to the high ultimate pH; however, most bacterial growth is not affected by a variation of pH from 5.5 to 7 (Gill & Newton, 1979). Rather, DFD meat's ability to spoil at a faster rate is thought to be due to the absence of glycogen in meat with a high pH (Gill & Newton, 1979). In normal pH meat, the presence of glycogen acts as the primary nutrient source for bacteria, and once glycogen has been depleted, bacteria utilise free amino acids as nutrients. This utilisation immediately releases by-products, such as ammonia and hydrogen sulphide, which can be responsible for the development of off-odours and off-flavours; typical symptoms of spoilage (Gill & Newton, 1979). The microorganisms in question include *Enterobacteriaceae*, Lactic acid bacteria such as *Lactobacillus* and *Leuconostoceae;* however, *Pseudomonas* spp. are dominant during the spoilage of refrigerated raw meat in an aerobic environment (Ercolini, Russo, Torrieri, Masi, & Villani, 2006).

Obviously, pH can have an influence on meat quality, but the bulk of research in this field falls heavily on studies conducted on beef, pork

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and mutton (Newton & Gill, 1981), with little research available on the spoilage of DFD game meat. Therefore, the aim of this investigation was to determine the effect pH has on colour and microbial quality of game meat, and to understand the spoilage process of game meat (originating from South Africa) with a high pH (DFD; pH > 6) compared to game meat samples with normal pH (< 6) over a 12-day storage period under aerobic conditions at low storage temperature (5 ± 1 °C), by observing pH, microbial (aerobic plate counts and *Enterobacteriaceae* counts) and colour changes.

2. Material and methods

2.1. Harvesting of animals and sample preparation

Twelve black wildebeest were harvested in April 2014 at Savanna game estate in Kimberly, South Africa. The black wildebeests were harvested according to the standard operating procedure with ethical clearance (approval number: SU-ACUM14-001SOP; Stellenbosch University Animal Care and Use Committee). Field exsanguinations were done within 2 min and carcasses were skinned, eviscerated, cleaned and chilled (muscle temperature < 6 °C within 24 h). After the development of rigor mortis (24 h post-mortem), carcasses were deboned and the left and right *Longissimus thoracis et lumborum* (LTL) muscles were removed from each carcass. Ultimate pH was measured in the LTL using a Crison 25 pH meter (Crison instruments S.A, Barcelona, Spain, purchased from Lasec, South Africa) calibrated before use with standard buffers of pH 7 and 4. Then, left and right-side LTL muscles were vacuum packed in high-barrier (moisture vapour transfer rate of 2.2 g/m^2 over 24 h at 1 atm, oxygen permeability of 30 cm^3/m^2 over 24 h at 1 atm and carbon dioxide permeability of 105 cm^3/m^2 over 24 h at 1 atm) polyethylene and nylon film vacuum bags (70 μm thickness) with a residual pressure of 5 mb (Multivac, Model C200; Sepp Haggenmuller, Wolfertschwenden, Germany) and frozen at −20 °C prior to analysis.

For the data analysis, the left and right sides of each carcass were considered an experimental unit because the variation between the muscles from left and right sides was considered to be negligible (Neethling, Suman, Sigge, & Hoffman, 2016). Vacuum packed LTL muscles were thawed for 24 h at 5 ± 1 °C in their vacuum-packed state. The following day packaging was first sprayed with 70% ethanol in order to minimise cross contamination from packaging to the LTL muscle. From each LTL muscle, five steaks were cut and were randomly allocated to a specific day of sampling in the refrigerated study. On each sampling day, steaks were further divided into three subsamples under a laminar flow cabinet for pH, colour and microbiological analyses. Steaks were kept on pre-labelled polystyrene trays over-wrapped with a low-density polyethylene wrap (LDPE; moisture vapour transfer rate of 585 g/m^2 over 24 h at 1 atm, oxygen permeability of 25,000 cm^3/m^2 over 24 h at 1 atm and carbon dioxide permeability of 180,000 cm^3/m^2 over 24 h at 1 atm) at 5 ± 1 °C in a GDC refrigerator with fluorescent lighting (sample temperature was monitored with an infrared thermometer, model no. TFC-42, Cape Instrument services Cc) for 12 days and sampled on days 0, 3, 6, 9 and 12.

2.2. pH measurements

Subsamples (1 g) were homogenised in a blender with 10 ml of a solution (pH 7.0) of 5 mM Sodium-Iodoacetate and 150 mM Potassium Chloride that was prepared prior to analysis. pH of the homogenate was measured (in duplicate) using a calibrated Jenway 3510 pH meter (Lasec, South Africa). Furthermore, to reduce temperature fluctuations, the homogenate was placed in ice and the pH meter was calibrated using standard buffers of pH 7 and 4 under the same cold conditions.

2.3. Colour measurements

Instrumental colour readings of LTL steaks (2 cm thick) were measured using a colour guide 45°/0° colorimeter (Catalogue number 6801; BYK-Gardner, Geretsried, Germany) equipped with an 11 mm aperture and using an illuminant/observer of D65/10°. The colorimeter was calibrated using the standards provided (BYK-Gardner). For day 0, the steaks were left to bloom for 40 mins before the readings were taken, however on day 3, 6, 9 and 12, no blooming was done as the samples were already exposed to oxygen due to the oxygen permeable packaging under which they were displayed. Instrumental colour variables measured were L* (representing darkness to lightness), a* (representing the green-red range), b* (representing the blue-yellow range) and the mean of five readings was used in the statistical analyses. Chroma (C*) and hue (H_{ab}) values were calculated from individual a* and b* values (Neethling et al., 2016) according to:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad \text{and} \quad H_{ab} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

2.4. Detection of microorganisms

2.4.1. Sampling procedure

Samples (25 g) were diluted 10-fold in buffered peptone water (CM1049, Oxoid, Hemisphere, England) and homogenised for 30s using a Seward stomacher 400 (Seward Medical UAC House, United Kingdom) at room temperature. Buffered peptone water was prepared according to the manufacturer's instructions.

2.4.2. Aerobic plate counts (APC) (ISO 4833:, 2003)

Serial 10-fold dilutions were prepared from the suspension using buffered peptone water. The pour plates with plate count agar (PCA; C6.500, Merck, Modderfontein, South Africa) were duplicated and left to solidify at room temperature. Thereafter, the plates were incubated fat 30 °C for 72 h before colonies were counted, using a Suntex colony-counter 570 (Lasec, South Africa). Plates were examined under a light to avoid mistaking particles of matter for colonies.

2.4.3. Enterobacteriaceae (ISO 21528-2:, 2004)

Serial 10-fold dilution was prepared and poured into duplicate plates, using violet red bile agar (VRBG; Oxoid, CM0485). Plates were left to solidify at room temperature before a second layer of VRBG was poured and left to solidify. Pour plates were then incubated at 37 °C for 24 h and all dark pink to red colonies were counted using a Suntex colony-counter 570 (Lasec, South Africa). Accurate counting was assisted with a bright light.

2.5. Statistical analysis

A receiver operating characteristic (ROC) curve was constructed in order to determine a pH cut-off point (a pH value that will ultimately be able to split samples into two pH groups) (García-Rey, García-Garrido, Quiles-Zafra, Tapiador, & Luque De Castro, 2004). In one group, the samples will be considered to be Normal in pH and the other, to be High in pH (DFD). Where applicable, an ANOVA was performed for all variables to determine significant differences over day 0, 3, 6, 9 and 12 and between pH categories. Where significant differences were found, a post-hoc Fisher's LSD test was done to determine the sources of variation. Where applicable, graphs and/or tables were generated from the means data (± S.E) (Miller, 1997).