QUANTIFICATION OF MUSCLE STRUCTURE BREAKDOWN DURING *POST MORTEM* AGING BY MEANS OF VIDEO IMAGE ANALYSIS

Phillip E. Strydom[1*], Hanlie D. Snyman[1], Kgantje W. Moloto[1] and Lorinda Frylinck[1]

[1] Agricultural Research Council, Private Bag X2, Irene, 0062, RSA

[#] Corresponding author (phone: +27-12-672-9340; fax: ; e-mail: pstrydom@arc.agric.za)

Abstract —**.Tenderness development in muscle is related to changes in the cytoskeletal structure which include detachment of fibers and sarcomeric breaks. Very often methods to quantify or relate to this process focus on micro-components, such as the degardation and formation of certain polypeptides and the measurement of myofibril fragments. This study focussed on a video image analysis (VIA) method to quatify structural changes on a more macro-structural basis. Variation in tenderness was improvised by different electrical stimulation applications, viz. No stimulation, 15 seconds, 45 seconds and 90 seconds low voltage stimulation (150 V, 17Hz, 5ms) which resulted in various rigor conditions. Warner Bratzler shear force (WBSF), myofibrillar length (MFL) and quantification of spaces due to detachments and breaks in muscle structure was measured. The latter was performed by VIA on staned longitudinally cut muscle sections. Samples were aged for 2 and 14 days. MFL was effective in describing changes in structure over duration of aging but did not relate well to variation in tenderness due to different stimulation treatments (i.e. rigor conditions and possible other structural changes such as contractions). The quantification of detachments and breaks by measuring the proportional areas of the spaces between fibers on muscle sections proved to be usefull to relate to variation in tenderness among treatments at a given aging time and over the duration of aging.**

Index Terms—**video image analysis, MFL, sarcomeric breaks, fibre detachment.**

I. INTRODUCTION

Muscle is a composite structure of contractile fibers attached to each other and organised by connective tissue. During *post mortem* aging, connective tissue mostly stays intact, while cytoskeletal components undergo changes such as detachment of fibers (or more specifically endomisium), breaks in sarcomeres and fiber contraction (e.g. with electrical stimulation or not)(Davey & Gilbert, 1969, Davey & Dickson, 1970, Gothard, Mullins, Boulware & Hansard, 1966, Olson & Parish, 1977, Will, Ownby & Henrickson, 1980). These changes are often related to degradation of certain proteins such as titin, nebulin, desmin and troponin-T (Ho, Stromer, Rouse & Robson, 1997). In addition, physical measurements such as myofibril length (MFL) or myofibrillar fragmentation index (MFI) are also often used to describe (or even quantify) structural break down and to relate these to meat tenderness (Moller, Vestergaard & Wismer-Pederson, 1973; Olson & Parrish, 1977). Quantitative changes described above are measured on an ultrastructural level, in structures which are probably too small to relate the sensorial perception of tenderness or even physical measurement by shear force.

In this study we have attempted to quantify the process of structural changes, i.e. fiber detachment and breaks in sarcomeres, by means of video image analyses (VIA) and relate these measurements to variation in Warner Bratzler shear force (WBSF).

II. MATERIALS AND METHODS

A. Animal material, trial lay-out and slaughter procedure

Eighty grain fed Bonsmara steers were divided into four groups of 10 animals so that the average weight and variation within each group were the same. The animals were slaughtered at a commercial abattoir and each group subjected to one of four electrical stimulation regimes applied, viz. One group was not stimulated (C) and the other three groups were stimulated for 15 (15S), 45 (45S) or 90 seconds (90S), respectively. Stimulation was applied by means of a low volt Jarvis system (150V, 17Hz, 5ms pulse width) five minutes after killing. Carcasses were dressed and chilled. Whole muscles portions of the loin (*M. longissimus lumborum*, LL) were sampled the day following slaughter, sub-sampled for measurement of WBSF, MFL or fiber detachments and breaks. For each test, samples were vacuum-packed and aged at 1 - 2°C for two or 14 days.

B. Sample analyses

Aged LL samples for WBSF were frozen at -20°C and then processed into two 30 mm steaks by means of a band saw. The frozen steaks were thawed at 4°C for 24 hours and cooked using an oven-broiling (Mielé, model H217, Mielé & Cie, Gütersloh, Germany) method with direct radiant heat (AMSA, 1995). The steaks were broiled at 260°C (pre-set) to 70°C internal temperature and cooled down to 18 °C. Six round cores (12.7 mm diameter) were removed from the steaks parallel to the muscle fibers (AMSA, 1995). Each core was sheared once through the center, perpendicular to the fiber direction, by a Warner Bratzler shear device mounted on an Universal Instron apparatus (Model 4301, Intsron Ltd, Buckinghamshire, England;.cross head speed = 200 mm/min) and the mean value of the six recordings used as a shear value.

MFL of LL was measured by means of VIA. Myofibrils were extracted according to Culler, Parrish, Smith and Cross (1978) as modified by Heinze and Bruggemann (1994). Hundred myofibril fragments per sample were examined and measured with an Olympus BX40 system microscope at a 400X magnification.

For measurement of fiber detachments and breaks, aged LL samples were frozen in liquid nitrogen. Sub-samples of 7mm x 4mm were mounted on a Cryotome disk and sections of 15 µm thickness were cut along the grain of the fiber with a Shandon Cryotome E and then fixed on a microscope slide. Sections were stained with Amaranth (Sigma A 1016-100G) and examined with an Olympus BX41 system microscope at 100X magnification. AnalySIS Life Science software package were used to measure the total area of muscle fibres and the total area of space between the fibres in a field of 0.57 mm².

C. Satistical analyses

Data were subjected to analysis of variance (GenStat® VSN International, Hemel Hempstead, UK; Payne, Murray, Harding, Baird & Soutar, 2007) with the four stimulation treatments as main effects. Means were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level (Snedecor & Cochran, 1980).

III. RESULTS AND DISCUSSION

Both stimulation (stimulation or not) and duration of stimulation had a significant effect on WBSF irrespective of duration of aging. Short (15S) and intermediate (45S) stimulation produced lower WBSF than C, while excessive stimulation (90S) showed tougher values compared to 15S and not significantly different from C. Considering the work of Ducasting, Valin, Schollmeyer and Cross (1985), Dransfield, Etherington and Taylor (1992) and Hwang and Thompson (2001) it could be argued that shorter stimulation advanced the onset of rigor (compared with C) sufficiently to trigger the action of calcium dependent proteinases (CDP), thereby causing proper initial and prolonged aging. With the same argument, longer stimulation (90S) onset of rigor was reached at very high temperatures in the present trial (Figure 1) possibly causing fast but inefficient aging due to excessive autolyses and inactivation of of calpains. In addition

MFL or MFI (myofibrilar fragmentation index) are often used to demonstrate muscle fiber degradation caused by the action of proteolytic enzymes as well as other actions causing destabilisation of the myostructure. Various researchers showed significant relationships between MFI or MFL and amount of aging, actual tenderness (shear force) and CDP activities (Olson, Parish & Stromer, 1976; Wahlgren, Olssen & Tornberg, 1997). In the present study samples aged for 14 days had shorter MFL's than samples aged for 2 days, which agrees with the previous authors. However, when measured within aging treatment, MFL at 2 and 14 days aging showed a poor relationships (r = 0.27 – 0.31) with WBSF at these points. From Table 1 it is clear that WBSF for C and 15S showed the biggest contrast, while MFL values grouped these two together in contrast to 45S and 90S. While Devine, Wahlgren and Tornberg (1999) agreed that MFL indicates the degree of proteolysis, they also found a lack of correspondence between MFL and tenderness differences under various *rigor* and conditions of muscle contracture. Strydom, Frylinck and Smith (2005) reported that MFL of stimulated and non-stimulated samples corresponded with WBSF at 14 days but not at 2 days *post mortem*. In both studies, other effects could have influenced tenderness, such as muscle contraction. Finally, King, Voges, Hale, Waldron, Taylor and Savell (2004) found no effect of low or high voltage stimulation on MFL during prolonged aging although stimulated meat was more tender at 1 and 3 days post-mortem.

According to Table 1, the quantification of the spaces developed between fibers as a result of fiber detachment and breaks between sarcomeric Z-bands agreed much more with variation in tenderness for both aging periods than MFL. C, being the toughest, showed the least detachment and sarcomeric break and 15S and 45 S the most. To some extent, the effect of overstimulation of 90S was also reflected in a tendency towards lower "white" spaces (breaks and detachment). Taylor and Frylinck (2003) used physical counts of breaks and detachments over various aging periods and found significant correlations between WBSF or taste panel scores and breaks at 7 and 21 days but no relationship with detachments. Mu-calpain were positively associated with fiber detachment. In our study we have not distinguished between breaks and detachments and while the correlation between "white" space and WBSF was fair (r = -0.53 to -0.55 for 2 days "white" space and 2 and 14 day WBSF) it was better than the relationship between MFL and WBSF. A possible explanation could be that the quantification of detachments and breaks describe changes on a more macro-structural basis than MFI or MFL and that MFI and MFL describe the aging process in such dimensions which

do not specifically relate to the mechanism of sensory evaluation of tenderness or physical shear force. This is particularly important in the light of the the work of Hatae, Yoshimatsu and Matsumoto (1990) suggesting that the size of structures which can be perceived by sensory analysis of meat could be limited to a minimum of 100µm, in other words the size of two muscle fibers.

Table 1: Means and standard errors of WBSF, MFL and areas of muscle fiber detachment and breaks for four electrical stimulation treatments.

	No stimulation (C)	Stimulation[e]		
		15S	45S	90S
Shear force:				
day 2 (kg)	6.93[c] (0.477)[d]	4.04[a] (0.432)	4.80[ab] (0.506)	6.02[bc] (0.640)
day 14 (kg)	4.13[c] (0.898)	2.31[a] (0.993)	3.23[ab] (0.993)	3.85[bc] (0.993)
Image analyses: myofibril length				
day 2 (µm)	32.8[a] (1.20)	31.8[a] (1.08)	41.0[b] (1.27)	43.1[b] (1.61)
day 14 (µm)	23.2[a] (1.46)	22.0[a] (1.32)	34.7[b] (1.55)	36.6[b] (1.96)
Image analyses: detachments and breaks				
% white area day 2	14.8[a] (0.803)	17.4[b] (0.726)	17.2[b] (0.851)	16.3[ab] (1.077)
% white area day 14	19.1 (1.15)	21.8 (1.04)	21.2 (1.22)	18.3 (1.55)

[abc] Means in the same row with different superscripts differ significantly (P<0.05)

[d] Values parenthesized are standard errors of means

[e] Duration of stimulation: 15S = 15 seconds; 45S = 45 seconds; 90S = 90 seconds

IV. CONCLUSION

Detachment of muscle fibers and sarcomeric breaks seem to be more related to variation in tenderness than MFL in scenarios where variation in rigor conditions influences tenderness at a specific point or throughout the aging process. Utilisation of VIA to quantify detachment and breaks measurement of spaces between muscle fibers seems promising to develop further. MFL procedure can still be used to show differences under the same processing situations, but in itself does not indicate absolute tenderness values.

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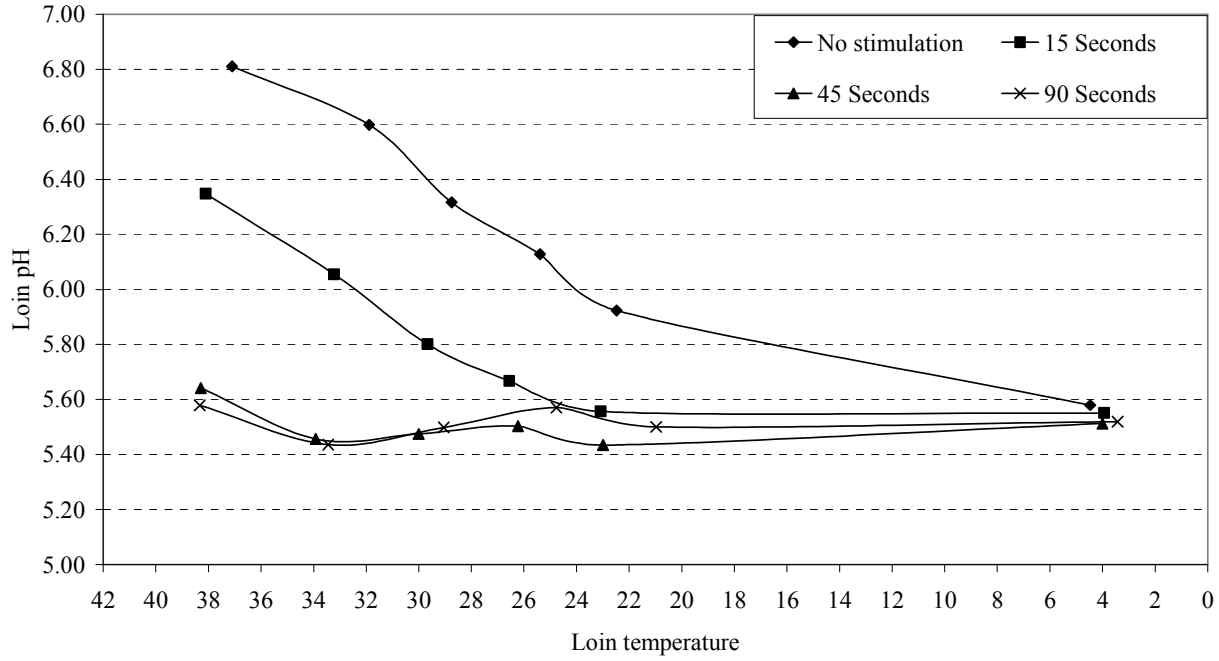

Figure 1: pH by Temperature decline rate for four stimulation scenarios (Recordings at 1 , 2, 3, 4, 5 and 24 hours post mortem)