THE EFFECT OF BREED AND AGEING ON THE SHEAR FORCE, PROTEIN SOLUBILITY AND MYOFIBRIL HYDROPHOBICITY

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Abstract – The study compares the differences in meat tenderness, total protein solubility, myofibril protein solubility, sarcoplasmic protein solubility and myofibril hydrophobicity between 5 South African beef breeds; *Bos indicus* (Brahman), Sanga type (Nguni), British *Bos taurus* (Angus), European *Bos taurus* (Charolais) and the composite (Bonsmara), and between 4 ageing periods. Ten animals per genotype, n=50 were used. The carcasses were split and the right sides were electrically stimulated and left sides not stimulated. Steaks were aged till 3 days (d) *post mortem* (pm) on polystyrene plates and till 9, 14 and 20 d pm in vacuum bags. Meat tenderness was measured using shear force and the total, myofibril and sarcoplasmic proteins solubility and myofibril hydrophobicity were determined using buffered salt solutions. Significant differences were observed between the breeds for the shear force, sarcoplasmic, myofibrillar and total protein solubility (P<0.0001). There were also significant differences between the ageing periods (P<0.0001). Correlation coefficients showed negative correlations between the shear force and total- (-0.51), myofibrillar- (-0.45), sarcoplasmic- (-0.49) protein solubility and surface myofibril hydrophobicity (-0.40).

Key Words – Breed, protein solubility, meat tenderness

I. INTRODUCTION

Meat tenderness is an important characteristic to indicate meat quality and it has been shown to be related to consumer satisfaction [1]. The type of beef breed has been found to greatly influence the characteristics of the raw muscle tissue and therefore of the finished product. Several studies have reported differences in the tenderness of meat between beef breeds [2; 3]. It is well recognized that the biochemical post-mortem processes are key-steps for meat tenderisation and that protein degradation is responsible for meat tenderness. Meat proteins are comprised of mainly the myofibrillar proteins, sarcoplasmic proteins, and connective tissue. Koohmaraie *et al*. [4] reported that proteolysis of myofibrillar and myofibrillar-associated proteins is responsible for meat tenderisation. Because these proteins are implicated in meat tenderness, information related to their denaturation pattern is of great importance

Protein hydrophobicity has been used to monitor subtle changes in chemical and physical state of protein and therefore it is a suitable parameter to estimate protein denaturation [5].

Because breed is an important factor that can influence the characteristics of the finished product; therefore, a comparative study of the most common breeds in South Africa was needed. It is also important to study the characteristics of some tenderness related proteins and try to establish if they are related to tenderness and breed differences. Therefore the objectives of this study were to: Evaluate the effect of breed and ageing on the protein solubility (sarcoplasmic, myofibrillar and total protein), shear force and evaluate the relationship between the shear force, protein solubility and surface myofibril hydrophobicity.

II. MATERIALS AND METHODS

The following genotypes were studied – *Bos indicus* (Brahman), Sanga type (Nguni), British *Bos taurus* (Angus), European *Bos taurus* (Charolais) and the composite (Bonsmara). Ten steers per genotype were purchased, n=50. The animals were fed on a feedlot diet for a period of between 90-110 d depending on their readiness. All animals were slaughtered, processed and sampled at the abattoir of the Animal Production Institute (Agricultural Research Council, Irene, Gauteng, South Africa). After exsanguination the carcasses were halved. The right sides were electrically stimulated for 20 s (400 V peak, 5 ms

pulses at 15 pulses/s) and entered the cold rooms (± 4°C) within 60 min after slaughter (ES treatment). The left sides were placed in a room with a controlled temperature of 10 °C for 6 hrs, after which they were placed in the cold rooms at ± 4 °C (NS treatment). The carcasses were sampled at the *m. longissimus dorsi* and two retail procedures were simulated for ageing of the steaks. The steaks were aged up to 3 d pm on polystyrene plates covered with polypropylene cling wrap (PP) at 6 °C in a display cabinet. The other steaks were aged up to 9, 14 and 20 d pm in vacuum bags at 1-4 °C in a cold room.

Shear force measurements

Shear force was determined using Instron fitted with a Warner Bratzler shear device. The steaks were prepared according to an oven broiling method using direct radiant heat [6]. Each steak was allowed to cool and 6 cylindrical cores were removed parallel to the grain of the meat. The cores were then sheared perpendicular to the fibre direction. The mean value of the six recordings was used as a shear value in kg.

Protein solubility measurements

Myofibrillar, sarcoplasmic and total protein solubility were determined according to the method of Joo *et al.* [7]. Sarcoplasmic proteins were extracted from 0.75 g muscle using 5 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). The samples were homogenized then left on a shaker at 4 °C overnight. Samples were centrifuged at 1500 g for 20 min and protein concentration in the supernatants was determined by the Biuret method. Total protein was extracted from 0.5 g muscle using 5 ml of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH7.2). The same procedures were used for protein determination as described above. Myofibrillar protein concentrations were obtained by difference between total and sarcoplasmic protein solubility. The protein solubility was expressed as mg/g of soluble protein.

Myofibril hydrophobicity

Myofibrils were prepared according to the method of Ouali *et al.* [8]. About 0.5 g of frozen muscle was homogenised in 5 ml of a solution at pH 6.5 containing 150 mM NaCl, 25 mM KCl, 3 mM MgCl2, and 4 mM EDTA. The homogenate was filtered to eliminate collagen. After 30 min stirring in ice, the extract was centrifuged at 2000 g for 15 min at 4 °C. The pellet was washed twice with 5 ml of a 50 mM KCl solution at pH 6.4 and once with 5 ml of 20 mM phosphate buffer at pH 6. The pellet was finally resuspended in the same phosphate buffer, and the protein concentration was adjusted to 5 mg/ml by the Biuret method.
One millilitre of myofibril suspension was heated, in a water bath at 70 °C for 60 min. The myofibril suspensions were immediately cooled for 10 min in ice and all samples were analysed for hydrophobicity using bromophenol blue.
The data were subjected to analysis of variance for a split plot design [9] with the five beef breeds (Angus, Bonsmara, Brahman, Charolais and Nguni) as whole plots and the four ageing periods (3, 9, 14 and 20 d pm) as sub-plots. Means for the interactions between sub-plot and whole-plot were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level of probability [10].

III. RESULTS AND DISCUSSION

Table 1 shows the effect of breed on the shear force and protein solubility, which included the total protein solubility, myofibrillar protein solubility and sarcoplasmic protein solubility and the myofibril surface hydrophobicity. There were significant differences between the breeds for the shear force ($P<0.0001$) and protein solubility ($P<0.0001$). The Warner Bratzler shear force was lower for the Nguni (3.86) and the Angus (4.06) and these breeds were regarded as producing more tender steaks, but the tenderness was similar to the Brahman and the Charolais (4.17 and 4.43 respectively). The Bonsmara steaks had the highest shear force values (4.73) and were regarded as producing the least tender steaks but the shear force was significantly similar to that of the Brahman and the Charolais.
The *Bos indicus* breed (Brahman) is known to produce less tender meat than the *Bos taurus* breed (Charolais and Angus) because the breed is believed to have a greater relative content of calpastatin which consequently contributes to greater inhibition of calpain resulting in tougher

meat [2]. The Sanga breed is known to produce high quality meat, comparative to the *Bos taurus* as reported by a number of studies [2]. The study did agree with previous findings but the *Bos indicus* breed was found to be similar in tenderness with the *Bos taurus* breed which did not agree with previous findings which reported the *Bos Taurus* to be more tender than the *Bos indicus* breed.

The total protein solubility was higher for the Nguni followed by the Brahman and the Charolais but were not significantly different from the Nguni. The Angus had lower total protein solubility. The myofibrillar protein solubility was higher for the Brahman, Charolais and the Nguni and lower for the Angus and the Bonsmara. The sarcoplasmic protein solubility was higher for the Bonsmara, followed by the Nguni which was similar to the Angus and Brahman. The Charolais had lower sarcoplasmic protein solubility which was not significantly different from the Angus and the Brahman. The Angus and Bonsmara had the lowest surface hydrophobic myofibrils and the Charolais had the highest. The Brahman was similar to the Charolais and the Nguni was similar to the Brahman and the Angus and the Bonsmara.

Table 2 shows the effect of ageing on the shear force, protein solubility and protein hydrophobicity. There were significant differences between the ageing periods for the Warner Bratzler shear force, total protein solubility, myofibrillar protein solubility, sarcoplasmic protein solubility and myofibril surface hydrophobicity. ($P < 0.0001$). The meat tenderness increased with ageing, day 20 was tenderer than day 14, followed by day 9 and day 3 was the least tender. The total protein solubility was lower for day 3 pm samples which were aged in display cabinet. The days 9, 14 and 20 pm steaks had similar total protein solubility. The myofibril protein solubility was higher for day 9 and 14 pm followed by day 20 pm and day 3 pm had lower myofibril solubility.

The sarcoplasmic protein solubility and the myofibril surface hydrophobicity increased with ageing, but the day 9 pm was similar to the day 14 pm. Table 3 shows a correlation matrix for correlation coefficients of shear force and total protein solubility, myofibril solubility, sarcoplasmic solubility and surface myofibril hydrophobicity. Relatively good correlations were found between the shear force and total protein solubility (-0.51), myofibril protein solubility (-0.45), sarcoplasmic protein solubility (-0.49) and surface myofibril hydrophobicity (-0.40).

IV. CONCLUSION

It is clear that there are not much differences between breeds in the tenderness, protein solubility and hydrophobicity measurements. The total, myofibrillar and sarcoplasmic protein solubility can act as a better indicator for meat tenderness than the myofibril hydrophobicity.

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Table 1: The effect of breed on shear force, total protein solubility, myofibril protein solubility, sarcoplasmic protein solubility and myofibril hydrophobicity of *M longissimus Dorsi* (LD)

	Beef breeds						
	Angus	Bonsmara	Brahman	Charolais	Nguni	SEM[1]	P-Value
Shear force	4.06^{b}	4.73^{a}	4.17^{ab}	4.43^{ab}	3.86^{b}	2.117	<.0001
Total protein solubility	176.697^{c}	183.67^{b}	186.35^{ab}	186.72^{ab}	188.41^{a}	14.109	<.0001
Myofibrillar protein solubility	105.13^{b}	107.99^{b}	114.53^{a}	116.42^{a}	116.85^{a}	15.354	<.0001
Sarcoplasmic protein solubility	71.57^{bc}	75.68^{a}	71.95^{bc}	70.297^{c}	72.72^{b}	6.947	<.0001
Myofibril hydrophobicity	79.32^{c}	78.20^{c}	86.00^{ab}	89.23^{a}	81.48^{bc}	14.669	<.0001

SEM[1] Standard error of means
^{a,b,c,d} Means within a row with different superscripts differ significantly (P<0.05)

Table 2: The effect of ageing on shear force, total protein solubility, myofibril protein solubility, sarcoplasmic protein solubility and myofibril hydrophobicity of *M longissimus Dorsi* (LD)

	Ageing					
	3 d pm	9 d pm	14 d pm	20 d pm	SEM	P-Value
Shear force	5.60^{a}	4.42^{b}	3.76^{c}	3.21^{d}	0.302	<0.0001
Total protein solubility	101.16^{b}	212.36^{a}	213.28^{a}	210.90^{a}	14.109	<.0001
Myofibril protein solubility	49.63^{c}	139.01^{a}	136.26^{a}	123.10^{b}	15.354	<.0001
Sarcoplasmic protein solubility	51.56^{c}	73.35^{b}	77.03^{b}	87.75^{a}	6.682	<.0001
Myofibril hydrophobicity	65.58^{c}	76.04^{b}	87.17^{b}	102.23^{a}	11.960	<.0001

SEM[1] Standard error of means
^{a,b,c,d} Means within a row with different superscripts differ significantly (P<0.05)

Table 3: Correlation matrix showing correlation coefficients of shear force and total protein solubility, myofibril protein solubility, sarcoplasmic protein solubility and myofibril hydrophobicity of *M. longissimus dorsi* (LD).

	Shear force	Total protein solubility	Myofibril protein solubility	Sarcoplasmic protein solubility	Myofibril hydrophobicity
Shear force	1	**-0.51**	**-0.45**	**-0.49**	**--0.40**
Total protein solubility	**-0.51**	1	0.97	0.74	0.45
Myofibril protein solubility	**-0.45**	0.97	1	0.54	0.36
Sarcoplasmic protein solubility	**--0.49**	0.739519	0.54	1	0.55
Myofibril hydrophobicity	-0.40	0.45	0.36	0.55	1