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PREDICTION OF SENSORY TENDERNESS OF BEEF MUSCLES HAVING DIFFERENT COLLAGEN CHARACTERISTICS USING WARNER-BRATZLER SHEAR FORCE

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Abstract: Present study was conducted to determine the certain physico-chemical attributes of selected muscles from four to six year old cross-bred cow and their correlation with sensory tenderness. Six years old cross-bred cow (cross between Holstein Friesian bull and Non-descript Indian cow) were utilized in this study and the following muscles were hot-deboned after low-voltage (100-110 V, 1.5 to 2 min) electrical stimulation of the carcasses: *serratus ventralis cervicis* (SEV), *supraspinatus* (SPS), *infraspinatus* (INF), *triceps brachii* (TRI), *longissimus thoracis et lumborum* (LNG), *psoas major* (PSM), *vastus lateralis* (VAL), *rectus femoris* (REF), *semimembranosus* (SEM) and *biceps femoris* (BIF). Each muscle was stored at $4\pm1^{o}C$ for 72 h and analyzed for physico-chemical and sensory attributes. Significant ($p<0.05$) difference in the collagen content (CC) and collagen solubility (CS) were observed between the selected muscles. The lowest Warner-Bratzler shear force (WBSF) was observed with PSM, while BIF had the highest WBSF value. The sensory evaluation revealed PSM, INF, SEV, REF and LNG having moderate or higher degrees of tenderness. The correlation coefficients of WBSF with sensory tenderness, overall acceptability, CC, CS, sensory amount of connective tissue were identical.
Keywords: Warner-Bratzler shear force; myofibril fragmentation index; cross-bred cow muscles; collagen; tenderness.

Introduction

Indian beef industry is mainly depend upon the slaughtering of spent animals after their productive life. Meat from such animals tends to be relatively tough and have more stable connective tissue component, which remains as residue after chewing. Tenderness of meat is one of the most perceived and highly desirable attributes among the consumers for beef. Consumers judge the quality and overall acceptability of beef products based on tenderness, which forms the primary economic factor for beef palatability (Boleman *et al.,* 1997). Meat tenderness generally decreases with animal age and it is mainly due to increasing of intramuscular connective tissue content. (Shorthose and Harris, 1990). Collagen characteristics may be the more important determinants of tenderness of muscles having higher collagen contents (Chriki *et al.,* 2012; Joo *et al.,* 2013). Sensory tenderness was found

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to be poorly correlated with the shear force values of bovine muscles which had largely different connective tissue contents (Cross *et al.,* 1973).

The link between intramuscular connective tissue content of muscles and cooked meat texture has been exemplified by the differential pricing of various cuts of meat (Purslow, 2014). Similar practices of beef carcass fabrication and value-tagging of meat at retail level is virtually non-existent in India, at least in the domestic market. Thus, consumer-ready packs of beef usually contain an assortment of different muscles. This results in failure by the traders to realize higher values for some of the muscles having potentially superior textural attributes. However, most of the studies on various quality attributes of beef was reported different muscles from the native and cross breeds in other countries but such studies in our conditions was limited. Hence, the present study was envisaged to determine and correlate certain physico-chemical attributes and sensory tenderness of selected muscles from mature cross-bred cow (Holstein Friesian (HF) X non-descript (ND)) and to identify those attributes that best predicted their sensory tenderness.

Materials and Methods

Six cross-bred cow with an age group of six from Cattle breeding farm, Thumburmuzhy, Kerala, India were utilized in this study. They were reared intensively under similar management practices with occasional periods of grazing. The animals were slaughtered as per scientific slaughter procedures at the Meat Technology Unit, Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala after 12-24 h fasting. The carcasses were electrically stimulated (100-110 V, 1.5 to 2 min) within 20 minutes of mechanical stunning and the following muscles were immediately harvested from each carcass by hot deboning, *viz. serratus ventralis cervicis* (SEV), *supraspinatus* (SPS), *infraspinatus* (INF), *triceps brachii* (TRI), *longissimus thoracis et lumborum* (LNG), *psoas major* (PSM), *vastus lateralis* (VAL), *rectus femoris* (REF), *semimembranosus* (SEM) and *biceps femoris* (BIF). Separable fat and epimysial connective tissue were removed. Each muscle was portioned for sensory evaluation, determination of Warner-Bratzler shear force and for analysis of other physico-chemical attributes. The muscle portions were packed in high density polyethylene (HDPE) pouches and aged for 72 h at 2-4°C (Samsung Digital Inverter Technology, India). After ageing, the pouches were transferred to a deep freezer and maintained at -18°C until further analysis which took place within one week of freezer storage. The samples were thawed at 4±1°C for 12 h prior to the assessment of parameters.

Collagen content

Collagen content of each muscle sample was determined as per Stegemann and Stadler (1967). Collagen content was determined by multiplying the hydroxyproline content with 7.25 and was expressed as per cent of fresh muscle weight.

Collagen solubility

Collagen solubility of each cross-bred cow muscles was determined as per Hill (1996) which was determined from the soluble hydroxyproline content of the sample. The collagen solubility was calculated by dividing the soluble collagen with collagen content and expressed as per cent of total collagen.

Myofibril fragmentation index

Myofibril fragmentation index of each muscle sample was determined after 72 h of ageing by the procedure outlined by Davis *et al*. (1980). Ten grams of 7-mm cubes of meat were added to 50 mL of 0.24 M cold sucrose and 0.02 M potassium chloride solution in a homogenization cup. After 5 minutes, each sample was blended for 40 seconds at full speed in a homogenizer (Polytron, PT 3100, Kinematica AG, Switzerland). The resulting homogenate was then filtered through a filter assembly consisting of a pre-weighed nylon cloth (250 µ pore size) in a glass funnel and glass stirring rod. The residue and cloth were blotted twice on absorbent towel immediately after stirring and then weighed. Fragmentation index was reported as weight of residue in grams times one hundred.

Warner-Bratzler shear force

Warner-Bratzler shear force of each beef sample was determined by the method outlined by Wheeler *et al.* (1997). Each muscle was cooked to an internal temperature of 80ºC (monitored using a probe thermometer), chilled overnight at 2-3ºC before coring. On the next day, three cores of 1.27 cm diameter were taken from each cooked meat along the longitudinal orientation of muscle fibres. These cores were kept at chiller temperature at 2-3ºC until they were sheared. Each core was sheared perpendicular to the muscle fibres on a Texture Analyzer (Model EZ-SX, Shimadzu Corporation, Kyoto, Japan) with a Warner-Bratzler shear attachment at a cross head speed of 200 mm/min. WBSF was expressed in Newton (N).

Sensory evaluation

The sensory evaluation of each cross-bred cow muscle was conducted by a semi-trained panel (n=10) consisting of scientist from the Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy. They were briefly told about the nature of the experiment without disclosing the identity of samples. Meat samples used were cut into approximately equal sizes (1.5 x 1.5 x 1.9 cm) and were cooked by indirect pressure cooking in small stainless steel boxes. Cooking was done under high flame till the first whistle and then kept to cook under low flame for 30 min.

All panelists received two cubes each of cooked beef coded with three digit numbers along with a score card. The panelists were asked to rate the samples for tenderness (1-extremely tough, 8=extremely tender), amount of connective tissue (1-abundant, 8-none) and overall acceptability (1-extremely unacceptable, 8-extremely acceptable) on an eight point hedonic scale (AMSA, 1983). Two fore-noon sessions were scheduled with a gap of 30-45 min between the sessions. Panelists were provided with filtered water to cleanse their palate between samples during sensory evaluation.

Statistical methods

Data recorded were analyzed statistically as per Snedecor and Cochran (1994) by repeated ANOVA measures. Results of sensory evaluation were analyzed by Friedman test. Correlation studies of physico-chemical and sensory attributes were performed by Pearson's and Spearman correlation coefficients respectively, using SPSS Software (Version 21.0).

Result and Discussion

Collagen is an abundant connective tissue protein and is a major contributing factor to variations in tenderness and texture. It is the connective tissue that most often contributes to less tender meat (Weston *et al.,* 2002). In the present study, there was moderate variability in the collagen content of different muscles (Table 1). PSM showed the lowest collagen content of 0.32±0.03 per cent which was similar to the value obtained by Kolczak *et al.* (2003). This was not significantly different from the collagen content of REF. Von Seggern *et al.* (2005) reported higher collagen content in REF from cross-bred cow carcasses. Rhee *et al.* (2004) also reported higher collagen content for REF from 14-16 months old beef carcasses. In the current study, higher collagen content was observed in BIF, the value being close to that reported by Von Seggern *et al.* (2005).

Soluble fraction of intramuscular collagen is a major determinant of the background toughness of meat. As the animal gets older, collagen cross-links get stabilized making it less soluble (Warriss, 2000). Among the cross-bred cow muscles PSM and LNG showed higher collagen solubility which differed significantly ($p<0.05$) from BIF which showed the lowest solubility for collagen (Table 1). There was no significant species difference in collagen solubility for majority of the selected muscles except REF. This could be due to similar rates of intramuscular collagen cross-linking in buffalo and bovine muscles as suggested by Valin *et al.* (1984). Silva *et al.* (1999) observed no correlation between soluble collagen and WBSF on different days of post-mortem ageing. In bovine muscles, soluble collagen has been found to decrease from 13.5 to 3.6 per cent as the age of animal increased (Cross *et al.,* 1973). Dransfield (1994) found that tenderness of beef from different age groups was primarily decided by the soluble collagen content. However, tenderness of beef from similar age groups was based on the collagen content. Difference in collagen solubility observed for BIF in the current study could be due to the difference in the management and activity levels of the animals. BIF was found to be the least tender muscle on sensory evaluation (see below). Hence BIF may be better suited for traditional methods of tenderization such as marination with weak organic acids or natural tenderizing agents to improve its acceptability.

Myofibril fragmentation index (MFI) of LNG reported the lowest MFI value where as SEM and BIF had significantly ($p<0.05$) higher values (Table 1). Myofibrils break into shorter segments at or near the Z-disk during the post-mortem tenderization of muscles (Davey and Gilbert, 1969). Rate and extent of post-mortem tenderization vary with muscle type and composition. The rate of pH fall due to glycolysis in combination with muscle temperature influences muscle shortening and proteolysis and there by meat tenderization (Guignot *et al.,* 1990).

Warner-Bratzler shear force (WBSF) is the most common objective biting or shearing type system used to measure meat tenderness. The peak force recorded in a Warner-Bratzler shear device has been the instrumental measure that has correlated most with sensory evaluation of toughness in cooked meat (Purslow, 2014). Among the cross-bred cow muscles BIF had the highest WBSF value followed by SEM. Bovine PSM muscle also showed the lowest WBSF. WBSF of SEV, INF, LNG and REF did not differ significantly. Bratcher *et al.* (2005) observed a WBSF value of 27.4 N for INF and 38.2 N for the lateral head of TRI which is similar to the results of the present study. Von Seggern *et al.* (2005) classified 39 beef muscles into three categories based on WBSF as tender (<37.6 N), intermediate (37.46 N to 47.96 N) and tough (>47.96 N). As per this classification, in the current study, buffalo PSM, INF, SEV, SPS, REF and LNG were tender, VAL, TRI and SEM were intermediate and BIF was tough. The bovine PSM, INF, SEV, REF and LNG were tender, SPS, TRI and VAL were intermediate and SEM and BIF were tough muscles. Sullivan and Calkins (2011), after a meta-analysis of various research papers, ranked *psoas major*, *infraspinatus*, *serratus ventralis cervicis, spinalis dorsi, multipidus dorsi* and *teres major* as tender muscles with

WBSF less than 3.9 kg. Major muscles classified in the tough group (>4.6 kg) were *biceps femoris*, *supraspinatus, vastus latralis*, *semitendinosus*, *Deep pectoral, rhomboidius, gluteus medius* and TRI.

Among selected muscles, PSM was the most tender and BIF the least tender muscle. Sullivan and Calkins (2011) reported that beef tenderness rating equal to or greater than six on an eight point hedonic scale had WBSF value less than 4.5 kg. In their study, PSM was rated the most tender and BIF as the toughest and INF, REF, TRI and SEV were classified as medium tender, and SPS and SEM were categorised as intermediate. Based on the sensory scores, PSM, LNG, INF, SEV and REF revealed a score of higher than 6.0 corresponding to the perception of moderate or higher degrees of tenderness. An intermediate range of sensory scores of 5 to 5.99 were shown by SPS, TRI and VAL. Sensory score of 4.99 and below corresponding to slightly tough and further increased degrees of toughness was shown by SEM and BIF.

The sensory score for the amount of connective tissue on an eight point hedonic scale (1- Extreemly abundant, 8- None), was found to be the lowest for BIF and highest for LNG. There was significant ($p<0.05$) difference for amount of connective tissue. Swelling and gelatinisation of intramuscular collagen during the process of moist cooking are highly dependent on the maturity of intramuscular cross-links in collagen. Collagen with more mature cross linking is less soluble and residue of collagen connective tissue is more perceived during sensory evaluation (Vasanthi *et al.* 2007).

Overall acceptability scores for cross-bred cow muscles, PSM had the highest and SEM had the lowest scores. INF had numerically higher score compared to TRI and REF and no significant difference was noticed between the PSM and LNG and SEM and BIF. The present results were in agreement with Robertson *et al.* (1986) and Lapitan *et al*. (2008).

Coefficients of correlation between sensory attributes and MFI, WBSF, collagen content and collagen solubility were determined by pooling the values of all the muscles (Table 3) (n=60). The WBSF was significantly ($p<0.01$) correlated with MFI, but a stronger correlation was found between WBSF and collagen content. The correlation coefficients for WBSF with collagen content and solubility were higher than those for MFI. This indicates that MFI not influenced by the amount and stability of intramuscular connective tissue matrix than WBSF. This is supported by the observation by Moller (1981) that WB peak force may be more correlated with the connective tissue component rather than the myofibrillar component of the shear deformation curve.

The WBSF was significantly ($p<0.01$) correlated with all the sensory characteristics, with the strongest correlation being observed with tenderness score ($r = -0.77$) followed by overall acceptability ($r = -0.50$) (Table 3). The correlation coefficients of MFI with all the three sensory attributes were significant ($p<0.01$) except overall acceptability but lower than those for WBSF. Similar observations were reported by Rhee *et al.* (2004). Other studies with bovine muscles have also shown a lower but significant correlation for sensory tenderness with MFI than with WBSF (Crouse *et al.,* 1991; Silva *et al.,* 1999).

Detectable connective tissue residue is the amount of insoluble connective tissue residue that remains even after thorough chewing, thus this parameter measured the relatively indestructible muscle component (Huff and Parrish, 1993). The correlation studies showed that when the solubility of collagen decreased, the sensory scores for amount of connective tissue (ACT) also decreased (which means higher connective tissue). ACT scores were also only weakly correlated with sensory tenderness ($r = 0.64$) and overall acceptability ($r = 0.51$) scores (Table 3). This was accordance with the previous reports (Rhee *et al.,* 2004), which have shown stronger correlation between ACT and sensory tenderness. The correlation between MFI and ACT was also relatively weak ($r = -0.31$). This indicates that MFI may not truly reflect the sensorily perceptible connective tissue characteristics of cooked beef.

In the current study, MFI was weakly correlated to sensory tenderness, but WBSF had strong correlation with the sensory tenderness and overall acceptability scores. This shows that WBSF can be an effective predictor of cooked beef tenderness and overall acceptability, while being strongly influenced by the amount and stability of the connective tissue component.

Conclusion

The present study was conducted on the muscles of cross-bred cow shows significant difference in their collagen content. WBSF was significantly and highly correlated with amount of connective tissue, collagen content, sensory tenderness and overall acceptability of the beef muscles than those for MFI. Hence, the study concluded that WBSF can be utilized as a predictor for sensory tenderness of beef muscles.

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Table 1. Physico-chemical attributes of selected cross-bred cow muscles

Muscle	Collagen content (per cent fresh basis)	Collagen solubility (per cent of collagen)	Myofibril fragmentation index	Warner-Bratzler shear force (N)
SEV	0.63 ± 0.03^{d}	3.53 ± 0.35^{ab}	811.67 ± 15.12^{bc}	28.12 ± 1.60^{b}
SPS	0.57 ± 0.03^{cd}	4.17 ± 0.34^{ab}	811.00 ± 39.75^{bc}	31.51 ± 0.50^{c}
INF	0.67 ± 0.04^{d}	3.56 ± 0.75^{ab}	782.17 ± 45.74^{b}	28.19 ± 2.05^{b}
TRI	0.51 ± 0.04^{bc}	3.72 ± 0.33^{ab}	813.33 ± 21.10^{b}	39.46 ± 0.83^{d}
LNG	0.42 ± 0.04^{ab}	4.58 ± 0.43^{b}	724.83 ± 35.60^{a}	29.45 ± 0.66^{bc}
PSM	0.32 ± 0.03^{a}	4.62 ± 0.52^{b}	767.50 ± 20.74^{ab}	23.59 ± 0.31^{a}
VAL	0.51 ± 0.09^{bc}	3.23 ± 0.64^{ab}	781.23 ± 36.46^{b}	38.80 ± 0.41^{d}
REF	0.38 ± 0.02^{a}	4.36 ± 0.82^{ab}	773.66 ± 30.69^{ab}	29.80 ± 1.00^{bc}
SEM	0.51 ± 0.03^{bc}	3.29 ± 0.30^{ab}	851.33 ± 19.27^{c}	51.56 ± 1.26^{e}
BIF	0.83 ± 0.06^{e}	2.32 ± 0.54^{a}	859.16 ± 31.82^{c}	56.30 ± 1.44^{f}

Mean of different attributes, bearing different lower-case alphabets in columns as superscripts indicates significant difference ($p<0.05$) (n=6). SEV-*serratus ventralis cervicis*, SPS-*supraspinatus*, INF-*infraspinatus*, TRI- *triceps brachii*, LNG- *longissimus thoracis et lumborum*, PSM-*psoas major*, VAL- *vastus lateralis*, REF- *rectus femoris*, SEM-*semimembranosus* and BIF- *biceps femoris.*

Table 2. Sensory attributes of selected cross-bred cow muscles

Muscle	Tenderness	Amount of connective tissue	Overall acceptability
SEV	6.20±0.07^{d}	5.22±0.14^{d}	5.66±0.27^{ab}
SPS	5.40±0.27^{cB}	5.57±0.21^{e}	5.66±0.27^{ab}
INF	6.41±0.12^{e}	5.47±0.03^{eB}	6.12±0.15^{c}
TRI	5.25±0.29^{bc}	4.91±0.05^{cB}	5.81±0.20^{bc}
LNG	6.89±0.08^{f}	5.70±0.13^{e}	6.53±0.12^{d}
PSM	7.22±0.12^{f}	5.65±0.07^{eB}	6.59±0.17^{d}
VAL	5.10±0.08^{bc}	4.90±0.02^{c}	5.79±0.14^{abc}
REF	6.20±0.15^{de}	4.92±0.14^{cB}	5.90±0.23^{bc}
SEM	4.89±0.21^{b}	4.40±0.07^{aB}	5.40±0.15^{a}
BIF	4.37±0.20^{a}	4.64±0.14^{bB}	5.40±0.21^{a}

Mean of different attributes, bearing different lower-case alphabets in columns as superscripts indicates significant difference ($p<0.05$) (n=6). SEV-*serratus ventralis cervicis*, SPS-*supraspinatus*, INF-*infraspinatus*, TRI- *triceps brachii*, LNG- *longissimus thoracis et lumborum*, PSM-*psoas major*, VAL- *vastus lateralis*, REF- *rectus femoris*, SEM-*semimembranosus* and BIF- *biceps femoris.*

Table 3. Coefficients of correlation of physico-chemical characteristics and sensory attributes of cross-bred cow muscles

	WBSF	MFI	CC	CS	TA	ACT	OA
WBSF		0.35**	0.42**	-0.44**	-0.77**	-0.68**	-0.50**
MFI			0.21	-0.37**	-0.33**	-0.30*	-0.25
CC				-0.69**	-0.50**	-0.18	-0.30*
CS					0.42**	0.29*	0.24
TA						0.64**	0.75**
ACT							0.51**
OA							

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level. Coefficients without any superscripts indicate no significant difference (n = 60). WBSF-Warner-Bratzler shear force, MFI-Myofibril fragmentation index, CC-Collagen content, CS-Collagen solubility, TA-Tenderness, ACT-Amount of connective tissue and OA- Overall acceptability.