Age related changes in lipid, collagen and hydroxypyridinium contents related to bovine Nelore Rhomboideus m. (*Bos indicus*) texture

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Abstract— **The aim of this work was to relate the influence of advance in age in relation to lipid and collagen and its crosslink hydroxypyridinium contents with texture in hump muscle, *Rhomboideus* m from bovine Nelore. Samples were taken in triplicate from 18, 24, 36, 48 months of age. The results for the lipid content were 16.77; 50.39; 3.76 and 14.22% for animals of 18, 24, 36, 48mo of age, respectively. These inconsistent values reflect the variability of the muscle in relation to season and management of the animals grown as free range livestock in a farm. However, the values for collagen and its crosslink increased with advance in age of the animals. Collagen contents were 0.84, 1.70, 5.99 and 6.67% and hydroxypyridinium concentration was 0.37, 0.42, 0.52 and 1.12 mol/mol of collagen for animals at the age of 18, 24, 36 and 48 months, respectively. Shear force values were 10.29; 5.79; 14.22 and 11.09 kgf for the age of 18, 24, 36 and 48 months, respectively. These results showed that in samples with high concentration of intramuscular fat, the presence of collagen and its crosslinks is not relevant but the total amount of lipid dictates the final texture irrespective of the animal age.**

Keywords— ***Rhomboideus*** **muscle, zebu, texture.**

I. INTRODUCTION

The Brazilian Association of Meat Exporting Industries [1] show the Brazilian cattle herd is the largest commercial herd in the world, beating the Indian and Chinese. It comprises about 80% of animals of Zebu (*Bos indicus*) and 20% of taurine breeds (*Bos taurus*). In 2009, the effective national cattle increased at 205.292 million head, an increase of 1.5% compared with the previous year, this data are Brazilian Institute of Geography and Statistics [2]. The developed humpback muscle, popularly known as cupim in Brazil, is unique to the zebu breed (Fig 1).

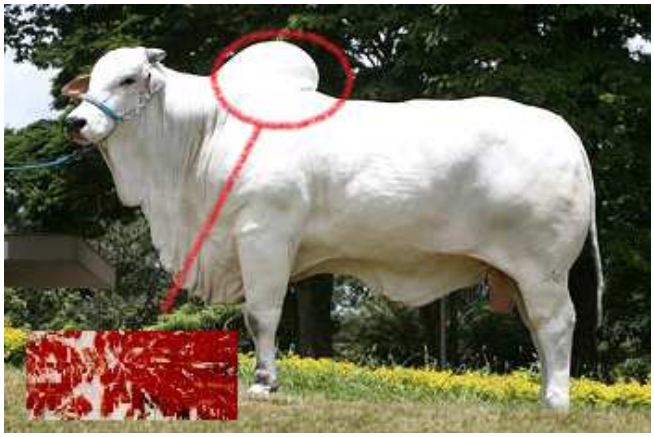

Figure 1 - Typical zebu nelore (*Bos indicus*) and the location of *Rhomboideus* m. Great Champion - Touro_Serro FIV da Bacaray-Brazil. [23]

It can be approx. 1.0% of the total cold carcass in weight and is much appreciated grilled as barbecue by Brazilians. It is believed that its biological origin was the necessity of the animal to have a supply of nutrients in order to resist long warm and dry season [3]. One of its characteristics is that, despite the visible presence of a high proportion of fat, it is relatively tough and to the best of our knowledge, there is no report available relating its chemical composition to its organoleptic qualities.

Meat texture sensation is dictated by several factors, including the amount of intramuscular fat, connective tissue, actomyosin complex, and its water holding capacity [4]. Collagen and its crosslinking are important factors to be considered [5, 6]. Nishimura et al [7] reported that abundant marbled intramuscular fat

is the main factor affecting the meat texture of Japanese Black cattle. Earlier studies indicated that marbling degree accounted for 3 to 10.0% of the variation in texture in a relatively small amount of beef intramuscular fat [8]. In this paper, we describe the influence of lipid content and of collagen and its crosslinking on the texture of hump muscle meat (*Rhomboideus m.*) having eye muscle (*Longissimus dorsi m.*) as control from the same animals with their advancing in age from 18, 24, 36 to 48 months.

II. MATERIAL AND METHODS

Animals: Twelve male zebus breed (*Bos indicus*) of 18, 24, 36 and 48 months old fed under native grasses raised at Paraná state region, Brazil, and slaughtered in a commercial abattoir (Jataizinho, PR, Brazil) were studied. The carcasses were kept refrigerated for 24 hours prior to analysis. Six samples of both *Rhomboideus* m. (RB) and *Longissimus dorsi* m. (LD) were excised from each carcass. Aponevrosis tissues were carefully removed by dissection and intramuscular samples were analysed.

Moisture, and protein concentrations were determined according to [9] The lipid extraction was quantitatively measured as described in [10].

Determination of Collagen and Hydroxylysylpyridinium (HP): Collagen was quantitatively evaluated by determining the amount of hydroxyproline (hypro) following Woessner technique [11]. Basically, 1.0g of ground intramuscular sample was hydrolyzed with distilled 6N HCl at 105ºC for 18 h. Hydrolyzate hypro concentration was determined by the reaction with pdimethylaminobenzaldehyde solution and colour intensity reading in spectrophotometer (Cintra 20, model GBC). The amount of collagen was determined by multiplying the colour intensity to 8.0 [12]. HP was analyzed by HPLC [13]). It consisted essentially in removing myofibrillar proteins with a 0.06M KCl treatment, centrifugation, and the precipitate was collected and dialyzed against several distilled water changes and finally lyophilized. Amounts of 50 mg of lyophilized samples were hydrolyzed in HCl 6.0N and non-crosslinking amino acids were initially separated on CF1 cellulose column as described [14] and adapted by [15]. After eluting the HP solution from the CF1 column with water, the samples were lyophilized and dissolved in buffer A (5.0% acetonitrile, heptafluorobutyric acid, HFBA). HP was eluted from the column by applying a gradient solution of buffer B (pure acetonitrile, HFBA) on a Shimadzu HPLC model RF-535 with a Supelco reverse phase column. The location of HP signal on the chromatogram was confirmed with an HP standard. The HP standard curve was obtained by using HP concentrations from 0.25 to 10 pmol considering collagen MW to be 3.38 x 105g of collagen and 429.1985 g for HP as stated in [16]

Meat texture: Texture was measured by Warner Bratzler shear force (WBSF) in an SMS Texture Analyser, TAXT2i model [17]. Before the measurement, samples (150-200g) were cooked in a vacuum-sealed plastic bag until the internal temperature reached 78-80ºC, cooled to 20ºC, and stored overnight at 4ºC [18, 6].

Optical microscopy: Meat samples were fixed in Bouin solution for 12h at room temperature, dehydrated and included in parplast as described by [19]. Samples were cut at 5µ size and stained in HE solution and picrosirius to observe collagen fibre distribution [18]. Samples were analysed by optical microscopy by Zeiss microscope, model Axiophot.

Statistical analysis: Results were processed using Statistica software package [21] and submitted to variance analysis and Tukey test in order to observe average differences.

III. RESULTS

Table 1 shows the moisture, lipid and protein contents of LD m. and RH m. of Nelore of 18, 24, 36 and 48 months of age. Also it is included the collagen and its crosslinking hydroxypyridynium and their texture measured by Warner Bratzler shear force. LD m. presented results as expected with the shear force increasing with age. However the texture for RH m. did not follow the same concept of the background toughness although collagen and HP contents also increased with advance in ageing

Table 1 : Hydroxylysylpyridinium, collagen, shear force, moisture, lipid and protein contents of LD m. and RB m. of Nelore of 18, 24, 36 and 48 months of age.

	18 mo.		18-24 mo.		24-36 mo.		36-42 mo.	
	RB	LD	RB	LD	RB	LD	RB	LD
Hydroxylysylpyridinium (mol/mol of collagen)	0.37 (±0.19) ^{a}	0.23 (±0.11) ^{a}	0.42 (±0.11) $^{a, b}$	0.24 (±0.10) ^{a,}	0.52 (±0.10) ^{b}	0.48 (±0.14) ^{b}	1.12 (±0.19) ^{c}	1.24 (±0.31) ^{c}
Collagen (%)	0.845 (±0.21) ^{a}	0.64 (±0.30) ^{a}	1.704 (±0.71) ^{b}	1.65 (±0.76) ^{b}	5.99 (±3.04) ^{d}	3.71 (±0.81) ^{c}	6.67 (±1.69) ^{d}	5.63 (±1.29) ^{d}
Shear force(Kgf)	9.33 (±2.85) ^{Aa}	8.89 (±1.53) ^{Aa}	5.81 (±1.32) ^{Bb}	8.05 (±1.93) ^{Ba}	12.01 (±1.74) ^{Cc}	10.62 (±1.82) ^{Cc}	11.20 (±2.28) ^{Cc}	7.13 (±1.78) ^{Dd}
Protein (%)	20.45 (±1.32) ^{b}	26.83 (±1.77) ^{a}	12.60 (±3.30) ^{c}	21.18 (±2.33) ^{b,d}	25.13 (±2.25) ^{a}	28.20 (±3.52) ^{a}	22.98 (±2.73) ^{b,d}	25.57 (±0.56) ^{a}
Lipids(%)	16.77 (±5.98) ^{Ab}	3.29 (±0.94) ^{Aa}	50.39 (±8.50) ^{Bc}	2.83 (±1.47) ^{Aa}	3.76 (±1.58) ^{Ca}	2.08 (±1.20) ^{Aa}	14.22 (±6.13) ^{Ab}	1.74 (±0.19) ^{Ba}
Moisture(%)	62,33 (±1.56) ^{b}	73,91 (±2.9) ^{a}	36,81 (±11.76) ^{c}	72,12 (±1.97) ^{a}	76,68 (±1.05) ^{a}	73,16 (±1.93) ^{a}	65,43 (±6.76) ^{b}	75,18 (±0.62) ^{a}

Different letters (column) indicate that there is significant difference between samples at 5% significance; same letters indicate no difference between the samples. Lowercase letters indicate differences between the two muscles (rows), capital letters indicate difference between the same muscles (columns).

Results showed a tremendous variability in its fat content and obviously in the moisture amount as it can be depicted in the Fig 2.

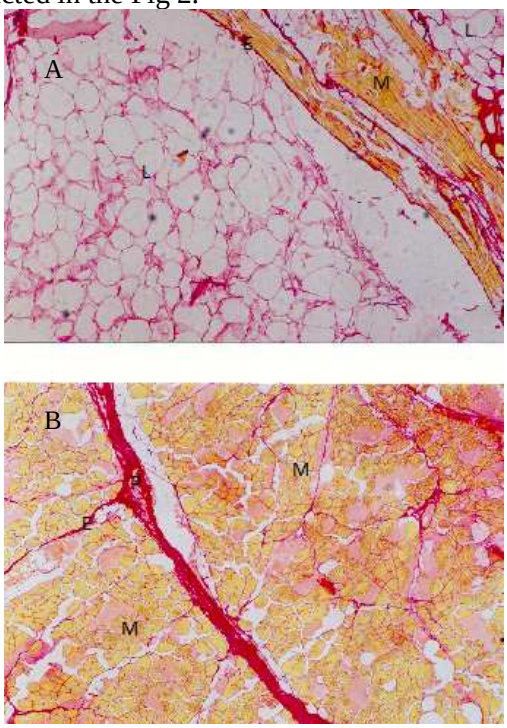

Figure 2A,B - Optical microscopy of the muscles Rhomboideus (A) and L. dorsi (B). -20x. Picrosirius staining. Red: collagen fibers, Yellow muscle cells, White: Fat cells . M: muscle cells, L: fat cells, P: perimysium. E: endomysium (Pedrao et al., 2009).

As it can be observed in Fig 2, there were differences in the distribution of fat and muscle cells in LD m. (A) and RH m. (B). There were far more fat cells in RH in relation to LD as demonstrated quantitatively in Table 1. Collagen sheaths were also observed within the perymisium (P) and endomysium.

IV. DISCUSSION

There are two reasons for the increase of meat texture with advance in age of the animal related to collagen. The increase amount of collagen content and also the stabilization of its crosslinking as observed in [5, 15, 6]. Although this condition can be observed in this experiment for LD m, the same is no observed for RH m. The WBSF values did not increase consistently with age as it would be expected and somehow observed in LD. Examining Table 1, it is observed the straight relationship between the lipid fraction content and texture being the higher amount of fat the tender is the meat measured by WBSF. In fact, taking into consideration the ratio lipid fraction and moisture, there was a tendency to relate the higher this ratio tender is the meat. The reason is that by measuring the texture in RB, the WB equipment needle did not find mechanical resistance as much as in LD because of excessive amount of fat it moves smoothly offering the result as tender. This is not a through sensation for consumers, since for its preparation as barbecue dish, after routine grilled cooking preparation, the hump

meat is tough and there was a need for several hours for preparation in a reasonable distance from the coal burning or cooking previously in a pressure cooker before grilling and obviously this precaution was not necessary for other kind of muscles. Fat fraction as the main reason for tender meat was also observed by [7] in Japanese Black cattle which contain a high content of intramuscular marble resulting in a remarkable tender meat.

V. CONCLUSION

Rhomboideus m from Indian Brahman origin is a unique muscle for its high content of fat although by measuring through Warner Bratzler Shear force equipment is in fact a tough meat and there is a need a special cooking treatment in order to make feasible for eating.

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