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Comparison of Proximate Chemical Composition and Texture of *cupim, Rhomboideus m.* and *lombo, Longissimus dorsi m.*of Nelore *(Bos indicus)*

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ABSTRACT

The proximate chemical composition of hump, known in Brazil as cupim*, Rhomboideus m. (RB), of Nelore (Bos indicus) aged 24 months revealed it to be a unique beef muscle. It presents a lipid fraction 14-fold as high as that of Longissimus dorsi m. (LD) taken from the same animal ($p<0.05$), the highest value reported so far. This was unequivocally observed by the histological evaluation. Proportionally more protein fraction and conversely less moisture were also observed in RB. Analysis of collagen and its crosslinking with hydroxylysylpyridinium (HP) showed there to be 22.9% more collagen and 14-fold as much HP in RB as in LD. Contrary to the expectations, the tenderness of fresh samples evaluated by Warner Bratzler shear force measurements led to values of 8.05 and 5.81 kgf for LD and RB, respectively ($p<0.05$). These results showed that the abundant fat in fresh RB acted as a lubricant for the needle penetration, irrespective of the quantity and quality of collagen fibres present.*

Key words: zebu breed, collagen, hydroxylysylpyridinium, meat tenderness, marbling fat

INTRODUCTION

There are approximately 180 million heads of beef cattle in Brazil; the majority is *Bos indicus*, which helps to make Brazil one of the major beef-producing countries in the world. The developed humpback muscle, popularly known as *cupim* in Brazil, is unique to the zebu breed. It can be approx. 1.0% of the total cold carcass in weight and is much appreciated barbecued 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 (Santiago, 1998) (Fig. 1). 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.

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Meat texture sensation is dictated by several factors, including the amount of intramuscular fat, connective tissue, actomyosin complex, and its water holding capacity (Avery and Bailey, 1995). Collagen and its crosslinking are important factors to be considered (Shimokomaki et al., 1972, Coró et al. 2002). Nishimura et al. (1999) 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 (Tatum et al., 1980). In this paper, we describe the influence of lipid content and of collagen and its crosslinking on the texture of *Rhomboideus m.* hump muscle meat. *L. dorsi m.* from the same animals is included in the study for comparison sake.

MATERIAL AND METHODS

Animals

Six zebu breed *(Bos indicus)* animals (24 months old) fed native grasses raised at North 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.

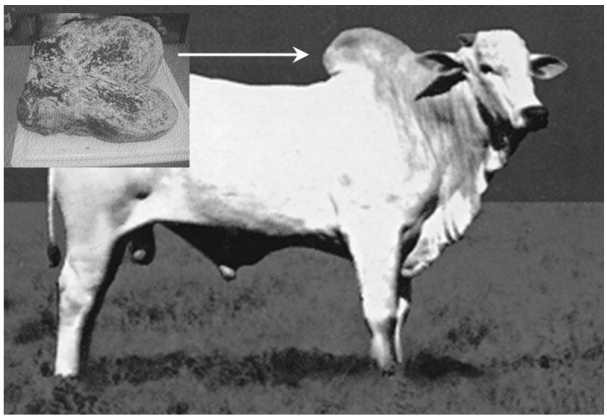

Figure 1 - Typical Nelore animals and the location of *Rhomboideus m*. The clear region of the muscle is fat and the brown one is muscle fibre.

Samples

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.

Basic chemical composition

Moisture, ash, and protein concentrations were determined according to AOAC (1995). The lipid extraction was quantitatively measured by the Bligh and Dyer technique (1959).

Determination of Collagen and Hydroxylysylpyridinium (HP)

Collagen was quantitatively evaluated by determining the amount of hydroxyproline (hypro) following Woessner technique (1961). Basically, 1.0g of ground intramuscular sample was hydrolysed with distilled 6N HCl at 105°C for 18 h. Hydrolysate hypro concentration was determined by the reaction with p-dimethylaminobenzaldehyde 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 (Kolar, 1980). HP was analyzed by HPLC (Avery, et al., 1996). 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 lyophilised. Amounts of 50 mg of lyophilised samples were hydrolysed in HCl 6.0N and non-crosslinking amino acids were initially separated on CF1 cellulose column as described in Skinner (1982) and adapted by Coro

et al. (2002). After eluting the HP solution from the CF1 column with water, the samples were lyophilised 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 10^5g of collagen and 429.1985 g for HP as stated in Bosselman et al., (1995).

Meat texture

Texture was measured by Warner Bratzler shear force (WBSF) in an SMS Texture Analyser, TAXT2i model (Bouton et al., 1971). Before the measurement, samples (150-200g) were cooked in a vacuum-sealed plastic bag until the internal temperature reached 78-80°C, cooled to 20°C, and stored overnight at 4°C. Subsequently, 50 meat cubes (ca. 1 cm^3) were cut from each sample and texture was measured (Young et al., 1994; Coró et al., 2003).

Light microscopy

Meat samples were fixed in Bouin solution for 12h at room temperature, dehydrated and included in parplast as described by Biscontini et al. (1996). Samples were cut at 5µ size and stained in HE solution and picrosirius to observe collagen fibre distribution (Junqueira et al., 1979). Samples were analysed by optical microscopy by Zeiss microscope, model Axiophot.

Statistical analysis

Results were processed using Statistica software package Statsoft™ (1995) and submitted to variance analysis and Tukey test in order to observe average differences.

RESULTS

Proximate chemical composition:

Table 1 shows the approximate chemical compositions of RB and LD muscles. These demonstrated a much higher incidence (12-15%) of intramuscular fat in RB than in LD. Conversely, LD presented twice as much moisture and 1.7-fold as much protein fraction as RB (Table 1). These results suggested that hump meat was more marbled than LD, being even more than most Japanese Black cattle muscles (Zambayashi et al., 1995) and had as much as 20% extractable lipids, ranking it as the fattest muscle so far reported.

Table 1 - Approximate % chemical composition of *Rhomboideus* m. (RB) and *L. dorsi* m. (LD) of 24-month old *Bos indicus* breed.

	RB	LD
Moisture	36.70[a] (±1.49)	73.34[b] (±1.77)
Ash	0.99[a] (±0.00)	0.99[a] (±0.00)
Lipid	48.82[a] (±6.80)	3.39[b] (±1.34)
Protein	12.60[a] (±2.70)	21.18[b] (±2.12)

[a,b]Within the same line means having different superscripts are significantly different ($p<0.05$).

Collagen content: collagen crosslinking and texture measurement

Quantitative analyses of collagen, HP and texture of both muscles are shown in Table 2.

Although RB presented 22.9 % more collagen and particularly approximately 14-fold as much crosslinked collagen as LD did, the latter was nearly 1.4-fold tough. These results were unexpected in absolute terms because the higher collagen values and crosslinking should lend a higher texture to the meat (Shimokomaki et al., 1972). One explanation lies in the higher level of marbling in RB, which offers lower resistance in WBS measurement (Table 2). On the other hand, in practice, hump muscle meat is perceived as tougher than LD after cooking by Brazilian consumers, and hence, it calls for longer cooking or harsher conditions such as pressure cooking to prepare it. The measurement of meat texture of hump samples with very high fat content by WBS force is not reliable as the lipid content acts as a lubricant and enables the WB needle to move smoothly through the sample. Obviously, this effect was not found in LD as shown in Table 2.

For most consumers, the texture sensation of meat prepared by common barbecue technique was due to the amounts of collagen and HP in meat (Table 2). From a practical cooking point of view, it is necessary to heat hump samples longer to break up collagen and its crosslinking. Results demonstrated that fresh Nellore hump muscle meat possessed a composition totally different from those of other beef skeletal muscles and that caution should be taken in measuring its tenderness and interpreting the results.

Table 2 - Quantitative analysis of collagen content (g/%), Hydroxylysylpyridinium (HP) (mo/mol) of collagen, and Warner Bratzler shear force (WBS) texture measurement (kg/F) of *Rhomboideus* and *L. dorsi* muscles of 24-month old *Bos indicus.*

Samples	Collagen	HP	WBS
Rhomboideus m.	12.40^{a} (±0.71)	0.452^{a} (±0.25)	5.81^{a} (±1.34)
L. dorsi m.	9.56^{b} (±0.62)	0.048^{b} (±0.015)	7.98^{b} (±1.93)

^{a,b}Within the same columns means having different superscripts are significantly different ($p<0.05$).

Light microscopy studies

Figures 2A and 2B show differences in the distribution of muscle (M) and fat (F) cells of LD and RB muscles. As quantitatively demonstrated in Table 1, there was far more F cells in LD than in RB muscles as expected. They show random distribution in LD in contrast to the localized areas of F and M cells in RB.

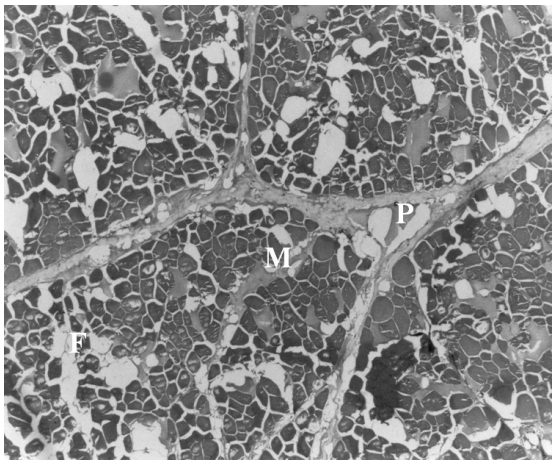

Figure 2A - Light micrograph of *Bos indicus L. dorsi* m. Note the distribution of fat (F) and muscle cells (M). P: Perymisium.x200.

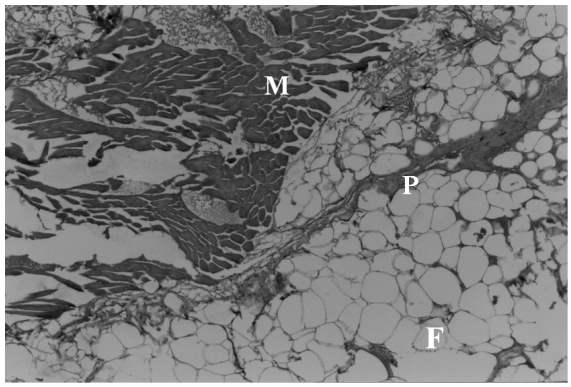

Figure 2B - Light micrograph of *Bos indicus Rhomboideus* m. Note the higher distribution of fat cells (F) in relation to muscle cells. P: Perymisium. x200.

The collagen fibres distribution at perymisium sheaths in both samples was also observed. Proximate chemical composition results demonstrated an incidence of intramuscular fat 12-15 fold as high in RB as in LD. Present results indicated that the Nellore hump muscle meat presented a totally different composition from those of other beef skeletal muscles, having unique sensory characteristics due to its abundant fat content.

RESUMO

A composição química percentual aproximada do *cupim m. Rhomboideus* (RB) derivado de Nelore *(Bos indicus)* de 24 meses de idade mostrou ser um músculo diferenciado. Há uma maior fração de material lipídico em RB chegando a ser 14 vezes proporcionalmente maior em comparação ao m. *Longissimus dorsi* (LD) ($p<0,05$) a maior quantidade de gordura relatada em amostras de carne em literatura científica. Esse fato foi também observado pela avaliação histológica. Há proporcionalmente maior concentração da fração protéica e menor quantidade de umidade em RB. O teor de colágeno foi de 22,9% mais concentrado em RB enquanto que a quantidade da sua ligação cruzada, hidroxilisilpiridinolina (HP) foi 14 vezes maior indicando que *cupim* seria mais rígido. Contrariando essa expectativa, a maciez da carne crua avaliada pelo texturômetro mostrou valores de 8.05 e 5.81 kg/F para LD e RB, respectivamente ($p<0.05$). Tais resultados mostram que em músculo contendo abundante gordura esta funciona como lubrificante para a lamina penetrar sem muita resistência através do *cupim* a despeito dar quantidade e qualidade das fibras de colágeno.

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