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Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics

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Abstract

Raw samples of 14 muscles: Mm. *biceps femoris* (BF), *quadriceps femoris* (CF), *diaphragm* (DI), *flexor digitorum* (FD), *gluteus medius* (GM), *infraspinatus* (IE), *longissimus lumborum* (LL), *longissimus thoracis* (LT), *psoas major* (PM), *pectoralis profundus* (PP), *semimembranosus* (SM), *semitendinosus* (ST), *sternomandibularis* (STER) and *triceps brachii* (TB) from four Swiss Brown (485±15 days old) young bull carcasses and weighing approximately 300 kg were evaluated for some chemical and physical properties. PM (2.11 kg) and DI (2.24 kg) were the muscles which had the lowest Warner–Bratzler shear force values, while PP (6.66 kg) had the greatest shear force ($P<0.05$). FD and IE muscles had the highest concentration of total collagen content while PM and DI had the lowest ($P<0.05$) contents, TB and IE muscles presented the highest insoluble collagen concentration while PM and LT had the lowest ($P<0.05$) contents. High positive correlation between total collagen content and Warner–Bratzler shear force of raw samples was found ($r=0.723$; $P<0.01$) and between insoluble collagen content and Warner–Bratzler shear force was ($r=0.661$; $P<0.01$). Significant differences ($P<0.05$) were observed among muscles for differential scanning calorimetry, sarcomere length, pH and colour parameters.

Keywords: Intramuscular connective tissue; Colour; pH; Sarcomere; Warner–Bratzler shear force

1. Introduction

The term ''quality'' includes many factors. Colour and texture are among the main ones, with texture the most important to the consumer. Tenderness and mechanical properties of meat are influenced by connective tissue, myofibrils and their interactions (Sacks, Kronick, & Buechler, 1988). Toughness due to the contractile proteins is determined to the decrease in sarcomere length, when rapid chilling causes ''cold shortening'' (Marsh, 1977). Collagen, the main component of muscle connective tissue, greatly influences beef toughness. Collagen has been said to contribute the so-called ''background'' toughness of meat. The general hypothesis is, therefore, that collagen is the major determinant of the texture of meat (when cold shortening is avoided) and that the subtle variations in texture are dependent on the quality rather than the quantity of collagen (Bailey & Light, 1989).

Dransfield, in 1977 showed a clear correlation between total collagen content and muscle toughness. Collagen concentration does not change significantly during growth until slaughter, but collagen solubility decreases with animal weight and age (Bailey & Light, 1989). Soluble collagen was significantly related to the contribution of connective tissue to toughness and tenderness differs among muscles from various anatomical locations (Cross, Carpenter, & Smith, 1973).

When a carcass is suspended, its weight generates tension in some muscles, ligaments and bones. Herring, Cassens, and Briskey (1965), Hostetler, Link, Landmann, and Fitzhugh (1972) and Quarrier, Carpenter, and Smith (1972) reported the effects of suspension methods on the tenderness of muscles, all these studies revealed a high correlation coefficient between sarcomere length and shear force in different muscles. Suspension

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by the Achilles tendon permitted the LL, GM, BF, ST and adductor muscles to shorten. When muscles shortened, there were corresponding decreases in sarcomere length, increases in fibre diameter, and decreases in tenderness (Herring et al., 1965).

Several workers in the past have attempted to estimate differences in tenderness and other properties among some beef muscles (Cross et al., 1973; Herring, Cassens, & Briskey, 1967; Mc Keith, De Vol, Miles, Betchel, & Carr, 1985; Seideman, 1986; Shorthose & Harris, 1990). The ultimate pH can be important in meat tenderness; Yu and Lee (1986) established that high pH meat (above 6.3) was most tender, followed by low (below 5.8) and intermediate (5.8–6.3) pH meats.

The crosslinking extent can be measured by determination of the thermal solubility of collagen (Bonnet & Kopp, 1986) or by differential scanning calorimetry (DSC; Le Lous, Flandin, Herbage, & Allain, 1982). DSC allows the study of phase transition and chemical reactions occurring within a muscle and can be successfully applied to measuring thermal transitions or denaturation temperatures of proteins. These transition temperatures are most commonly specified as temperature of maximum peaks, enthalpy changes (ΔH) or the amount of specific heat involved in protein denaturation (Kijowski & Mast, 1988). Intramuscular connective tissue (IMCT) of bovine BF was reported to have a thermal transition temperature significantly higher than that of the *longissimus dorsi* muscle, due within one animal, the stability of collagen vary from one site to another (Field, Pearson, & Schweigert, 1970).

Most studies on the structure and composition of the meat have focused on the loin and the hindquarter of the carcass. Other muscles with lower commercial value have not been studied except by Herring et al. (1967); Mc Keith et al. (1985), Seideman (1986), and Shorthose and Harris (1990).

The main objective of this study was to evaluate total and insoluble collagen content, sarcomere length, differential scanning calorimetry, pH and colour parameters of 14 beef muscles and their relationship with meat toughness.

2. Materials and methods

2.1. Materials

Meat was obtained from four Swiss Brown (485±15 days old) young bull carcasses, weighing (cold carcass) approximately 300 kg, with similar genetic backgrounds and fed with the same diet. The carcass was suspended by Achilles tendon and after washing was placed in the cooler room at 1±1 °C and wind speed of 2 m/s. After 24 h post-mortem, 14 muscles were removed from each carcass [*biceps femoris* (BF), *quadriceps femoris* (CF), *diaphragm* (DI), *flexor digitorum* (FD), *gluteus medius* (GM), *infraspinatus* (IE), *longissimus lumborum* (LL), *longissimus thoracis* (LT), *psoas major* (PM), *pectoralis profundus* (PP), *semimembranosus* (SM), *semitendinosus* (ST), *sternomandibularis* (STER) and *triceps brachii* (TB)]; each muscle was trimmed of all visible fat and epimysium and was divided in homogeneous samples of 150 g for each analysis, they were vacuum packed, frozen (−30 °C), and stored at −20 °C.

2.2. pH

The pH of all muscles was measured at 24 h post mortem; about 3 g of muscle was homogenised in 20 ml distilled water for 15 s. The measurement was done using a Crison pH-meter with a combined glass electrode.

2.2.1. Colour

Colour objective measurement (CIE L^*, a^* and b^*) was measured at the surface of fresh muscle samples using a reflectance spectrophotometer (Minolta CM 2002, Japan). Prior to colour evaluation, steaks were allowed to oxygenate for approximately 30 min at 1 °C, in order to stabilise the colour on air exposure. The average value for each sample was the mean of 25 determinations.

2.3. Texture

Muscle samples of raw meat were removed from each muscle for textural properties, stress at maximum was measured with a Texture Analyser model TA-XT2i from Stable Micro Systems (Surrey, UK). Meat samples, a minimum of 10 cores, of rectangular cross-section of about 6 cm long×1 cm high×1 cm wide were placed inside the Warner–Bratzler shear blade to be sheared perpendicularly to the long axis of the muscle fibres.

2.4. Collagen

Collagen concentration was determined from the hydroxyproline content according to the method of Bergman and Loxley (1963) modified by Bonnet and Kopp (1986), and expressed as mg of hydroxyproline per g of wet muscle.

2.5. Collagen solubility

Collagen solubility was determined by the method described by Bonnet and Kopp (1992) where the heat stability of collagen was assessed from the residual collagen content obtained after 2 h heat treatment at 90 °C in an isotonic buffer solution (0.02M Tris–HCl buffer pH 7.5 containing 0.23 M NaCl) and elimination of the dissolved collagen fraction. Hydroxyproline content of

the insoluble fraction was determined as described earlier for total collagen and was expressed as mg of hydroxyproline per g of muscle.

2.6. Differential scanning calorimetry

Extraction of IMCT and DSC were performed using the method described by Beltrán, Galán, Jaime, and Roncalés (1994) with a DSC V4.OB Dupont 2000 with indium and pure water as calorimetric standards. Samples of collagen (3 mg) sealed in stainless steel pans were heated from 25 to 90 °C at 2 °C/min. Ten samples were measured from each muscle. Thermal curves were analysed for the temperature (°C) of transition T_2 (maximum).

2.7. Sarcomere length

Sarcomere length was evaluated twenty four hours post mortem, small cubes of about 3 g taken from all muscles studied were fixed by immersion for 1 h in glutardialdehyde solution (2.5% v/v in phosphate buffer pH 6.5). Four bundles of 2–3 fibres were removed from them, and the length of 10 consecutive sarcomeres was measured in 10 randomly selected fibre fragments using an inmersion objective (×100) and a graduated ocular (×10) on a phase contrast Nikon microscope model L-ke.

2.8. Statistical analysis

Statistical analysis was carried out with SPSS (Statistical Package for the Social Sciences) for windows version 10.0.6 (1999). Significance of differences among muscle samples was determined by analysis of variance (ANOVA) using the least square difference method (Duncan test) to find out differences within studied factors. Differences were considered significant at the $P<0.05$ level. Correlation analysis was used to study the relationships between variables. A canonical discriminant test was made using a Mahalanobis method and applied to groups within the studied factors performed a matrix structure according to the physical and chemical characteristics studied [WBSF, T_2 (maximum), total and insoluble collagen, sarcomere length, pH and colour parameters] and to discover which variables were responsible for this distribution.

3. Results and discussion

3.1. Texture measurements

Results of physical and chemical properties of 14 beef muscles are given in Table 1. Warner–Bratzler shear force values ranged from 2.11 kg PM to 6.66 kg PP. PP, STER and FD had the highest value of shear force. PM and DI were the most tender muscles; this disagreed with the results of Boccard (1981) for DI muscle (4.2 kg). However, it is not clear if raw or cooked meat was used in that study; furthermore, the author did not indicate if DI tendinous lamina was removed before the analysis. Different laboratories used different methods in the past, which makes comparison of results across studies difficult (Wheeler, Shackelford, Johnson, Miller, Miller, & Koohmaraie, 1997). Shear force on raw meat is mainly reflecting background or collagen toughness, whereas shear force on cooked meat may be considered a measure of myofibrillar toughness (De Smet, Claeys, Buysse, Lenaerts, & Demeyer, 1998).

3.2. Total and insoluble collagen content

Total and insoluble collagen contents of the muscles showed a wide range of values with significant differences ($P<0.05$) Table 1. Total collagen content of PM was the lowest and FD had the highest total collagen content. The results for total collagen concentration of the muscles differed a little from other reports. Cross et al. (1973) reported similar values of total collagen for BF and ST but lower values for *longissimus* muscle; Mc Keith et al. (1985) found lower total collagen contents in the PM, LL and LT, than in IE, TB and BF, which is in agreement with our results. Seideman (1986) reported the same distribution of muscles and De Smet et al. (1998) found similar results too. The variation of the results obtained in the literature could be explained by underestimation of hydroxyproline content, when samples were prepared by freezing, or overestimation when samples were prepared by other methods.

The content of insoluble collagen differed significantly ($P<0.05$) between muscles (Table 1); PM presented the smallest concentration, while the TB had the highest content. The collagen solubility percentages vary from 16.24% to 55.32%. The PM and FD had the highest collagen solubility while SM and BF the lowest. These results are in agreement with the results of Seideman (1986), who reported the lowest collagen solubility in ST, SM and BF and the highest in PM. However, they differed in their solubility percentages, since our values for PM were higher. The high solubility in LD and PM is consistent with the results of Campo et al. (2000), who reported similar high solubility values in *M. longissimus dorsi*. This high solubility could probably be due to the solubilisation method used. Most authors use 77 or 70 °C for 65 or 70 min, while Bonnet and Kopp (1992) method uses 90 °C for 2 h. Breed type, could be another possible explanation because young animals (<16 months) fed high grain diets produce meat with collagen solubility values approximately 50%; greater than those fed corn silage (Rompala & Jones, 1984). In other research Crouse, Cross, and Seideman (1985) found that meat from animals on a high-energy diet tended to have

Table 1
Rank of 14 bovine muscles by Warner–Bratzler shear force, total collagen, insoluble collagen, sarcomere length and differential scanning calorimetry (DSC)

Muscle rank	Warner–Bratzler shear force (kg)	Total collagen (mgHyp/g wet tissue)	Insoluble collagen (mgHyp/g wet tissue)	Sarcomere length (μm)	DSC (T_2 °C)
1	*psoas major*	*psoas major*	*psoas major*	*l. lumborum*	*flexor digitorum*
	2.11a±0.39	0.31a±0.04	0.18a±0.01	1.57a±0.3	59.36a±0.88
2	*diaphragm*	*l. thorecis*	*l. thoracis*	*gluteus medius*	*l. thoracis*
	2.24a±0.90	0.51b±0.04	0.38b±0.03	1.59ab±0.2	60.37b±0.92
3	*l. thoracis*	*diaphragm*	*diaphragm*	*biceps femoris*	*infraspinatus*
	2.29a±0.91	0.53c±0.08	0.39b±0.10	1.6ab±0.2	60.60bc±0.36
4	*l. lumborum*	*gluteus medius*	*l. lumborum*	*quadriceps femoris*	*l. lumborum*
	2.35a±0.59	0.59d±0.05	0.42c±0.03	1.71b±0.4	60.70c±0.72
5	*gluteus medius*	*semimembranosus*	*gluteus medius*	*diaphragm*	*psoas major*
	3.68b±0.51	0.60de±0.06	0.45d±0.04	1.84c±0.2	60.87cd±0.46
6	*semimembranosus*	*l. lumborum*	*semimembranosus*	*triceps brachii*	*gluteus medius*
	4.10c±0.79	0.61e±0.09	0.49e±0.02	1.87c±0.1	60.89cd±0.6
7	*infraspinatus*	*quadriceps femoris*	*flexor digitorum*	*semimembranosus*	*triceps brachii*
	4.24c±0.93	0.68f±0.09	0.51f±0.06	1.93cd±0.2	61.13d±0.56
8	*quadriceps femoris*	*biceps femoris*	*quadriceps femoris*	*l. thoracis*	*sternomandibularis*
	4.72d±0.89	0.72g±0.05	0.52f±0.06	1.95cd±0.5	61.45e±0.84
9	*semitendinosus*	*semitendinosus*	*sternomandibularis*	*semitendinosus*	*pectoralis profundus*
	4.79d±0.58	0.77h±0.09	0.57g±0.06	2.05d±0.2	61.64e±0.82
10	*triceps brachii*	*sternomandibularis*	*biceps femoris*	*infraspinatus*	*semimembranosus*
	5.42e±0.82	0.81i±0.07	0.60h±0.04	2.21e±0.3	61.66e±0.83
11	*biceps femoris*	*triceps brachii*	*semitendinosus*	*flexor digitorum*	*quadriceps femoris*
	5.46e±0.84	0.94j±0.08	0.64i±0.03	2.39f±0.2	61.68e±0.9
12	*flexor digitorum*	*pectoralis profundus*	*pectoralis profundus*	*sternomandibularis*	*biceps femoris*
	5.93f±0.92	0.96k±0.09	0.71j±0.04	2.55g±0.2	62.02f±0.51
13	*sternomandibularis*	*infraspinatus*	*infraspinatus*	*pectoralis profundus*	*semitendinosus*
	6.32g±0.72	0.98k±0.05	0.76k±0.08	2.65g±0.3	62.24f±0.47
14	*pectoralis profundus*	*flexor digitorum*	*triceps brachii*	*psoas major*	*diaphragm*
	6.66h±0.66	1.15l±0.03	0.78l±0.02	3.42h±0.3	62.32f±0.39

Means within a column with a common letter are not significantly different ($P<0.05$). Hyp = hydroxyproline. T_2 the peak maximum of temperature of transition.

greater quantities of total collagen and similar quantities of insoluble collagen. This suggested that the total amount of connective tissue is associated to the animal diet.

3.3. Sarcomere length

A wide range of sarcomere lengths (1.57–3.42 μm) was found among muscles (Table 1). Differences were statistically significant ($P<0.05$). The PM and the LL had the longest and shortest sarcomere lengths, respectively. These results are in agreement with the results of Herring et al. (1965), Hostetler et al. (1972) and Mc Keith et al. (1985) for carcasses suspended in the traditional manner (Achilles tendon).

3.4. Differential scanning calorimetry

The results of DSC (T_2 °C) ranked from lowest to highest are shown in Table 1. Significant differences T_2 ($P<0.05$) among muscles were evident; FD (59.36 °C) had the lowest T_2 temperature and DI (62.32 °C) had the highest denaturation temperature value. The low T_2 observed in FD muscle was related to the high percentage of soluble collagen of this muscle when compared to any other muscle, despite its high collagen content. Furthermore, in young animals, in which the collagen contains fewer stable crosslinks, the shrinkage tends to lead to a decrease in toughness (Ledward, 1984). The DI muscle showed a different behaviour, the high transition temperature T_2 reached was a consequence of the smaller solubility that possesses of the collagen of this muscle.

3.5. Colour and pH

Significant differences were found among muscles for colour and pH (Table 2). GM (5.42) presented the lowest pH and STER the highest (5.77). PP and DI had the lowest brightness (L^*), 36.1 and 37, respectively. DI had the highest a^* value (18), which was probably due to the fact that the diaphragm has a high myoglobin concentration. This observation was also made by Boccard (1981), who reported that the pigment level of DI was 9.06 mg/g fresh tissue, the highest in five muscles from bull calves. Furthermore, Talmant and Monin (1986)

Table 2
Measures of pH and colour (L^* a^* b^*) of 14 bovine muscles

Muscle rank	Muscle	PH	L^*	a^*	b^*
1	*gluteus medius*	5.42a±0.06	41.2f±1.6	14.2d±1.4	15.1d±1.2
2	*longissimus lumborum*	5.49b±0.13	39.9e±1.1	15.0ef±1.3	14.0b±0.9
3	*semimembranosus*	5.50b±0.13	41.6g±1.5	15.4g±1.2	13.7b±1.2
4	*biceps femoris*	5.52bc±0.04	39.8de±1.5	10.5a±0.7	13.2a±1.1
5	*semitendinosus*	5.52bc±0.05	43.7h±1.4	12.5b±1.2	14.1b±0.8
6	*triceps brachii*	5.53bc±0.05	39.4d±1.2	14.8f±1.2	13.8b±1.1
7	*quadriceps femoris*	5.57cd±0.14	39.7d±1.3	12.3b±0.7	14.0b±1.1
8	*psoas major*	5.58de±0.09	38.7c±1.6	15.2g±1.2	14.0b±1.1
9	*pectoralis profundus*	5.62def±0.20	36.1a±1.3	15.8h±0.9	15.4d±0.6
10	*infraspinatus*	5.63ef±0.04	39.4de±1.6	13.4c±1.5	13.2a±0.9
11	*diaphragm*	5.65f±0.14	37.0b±0.9	18.0j±0.7	13.2a±1.1
12	*longissimus thoracis*	5.66f±0.06	41.0f±1.2	14.5ef±1.3	14.6c±0.9
13	*flexor digitorum*	5.67f±0.13	39.9e±1.4	17.4i±1.1	13.1a±1.0
14	*sternomandibularis*	5.77g±0.16	39.7de±1.4	14.3de±1.3	12.8a±0.9

Means within a column with a common letter are not significantly different ($P<0.05$).

studied 18 muscles from bovine carcass and found that haem iron concentration of DI was the highest, in good agreement with our results.

3.6. Correlations between variables

Table 3 shows the correlation coefficients among nine variables. High positive correlations between WBSF and total collagen content ($r=0.723$; $P<0.01$), and insoluble collagen content ($r=0.661$; $P<0.01$) were found. These correlations agreed with De Smet et al. (1998) who reported high correlations between shear force values and total collagen content on raw meat and Destefanis, Barge, Brugiapaglia, and Tassone (2000) who reported high correlations between WBSF and cooked meat. A weak correlation between sarcomere length and WBSF was observed (0.096; $P>0.05$). It is interesting to note that the correlation between total and insoluble collagen was 0.778 ($P>0.01$). This high correlation agreed with Crouse et al. (1985) who reported positive correlations between total collagen and insoluble collagen. The correlation between DSC and total collagen content for all 14 muscles was −0.208, and 0.123 for insoluble collagen; these low correlations are in agreement with the preliminary results of Torrescano, Sánchez, Roncalés, and Beltrán (2000). Low correlations were observed among the rest of parameters analysed.

3.7. Discriminant analysis

The results of the canonical discriminant analysis are presented in Fig. 1. This analysis showed that 75.3% of total variation is explained by Function 1 and 12.6% by Function 2, making a cumulative of 87.9%. The score plot shows the location of the muscles in the discriminant space, in which it can be seen that the scores are arranged and discriminated according to texture and collagen characteristics. Function 1 was capable of stressing the differences between those muscles considered as tender according to our results (PM, DI, LT, LL, GM, SM and CF) and those we classified as tough (IE, TB, PP, FD, STER, ST and BF). Function 1 (horizontal axis) discriminated and arranged leg and

Table 3
Correlation coefficients of 14 bovine muscle traits[a]

	WBSF	Tc	Ic	SL	DSC	L^*	a^*	b^*	pH
WBSF	1								
Tc	0.723**	1							
Ic	0.661**	0.778**	1						
SL	0.096	0.017	−0.201**	1					
DSC	0.083	−0.208**	0.123**	−0.104	1				
L	−0.075	−0.102	−0.001	−0.262**	−0.039	1			
a	−0.162**	0.033	−0.272**	0.237**	−0.227**	−0.370**	1		
b	−0.070	−0.135	−0.040	−0.050	0.017	0.020	−0.033	1	
pH	0.083	0.163**	0.004	0.234**	0.038	−0.196**	0.164**	−0.192**	1

[a] Warner–Bratzler shear force (WBSF); total collagen (Tc); insoluble collagen (Ic); sarcomere length (SL); differential scanning calorimetry (DSC); colour values L^*, a^*, b^*; pH.

** Level of significance ($P<0.01$).

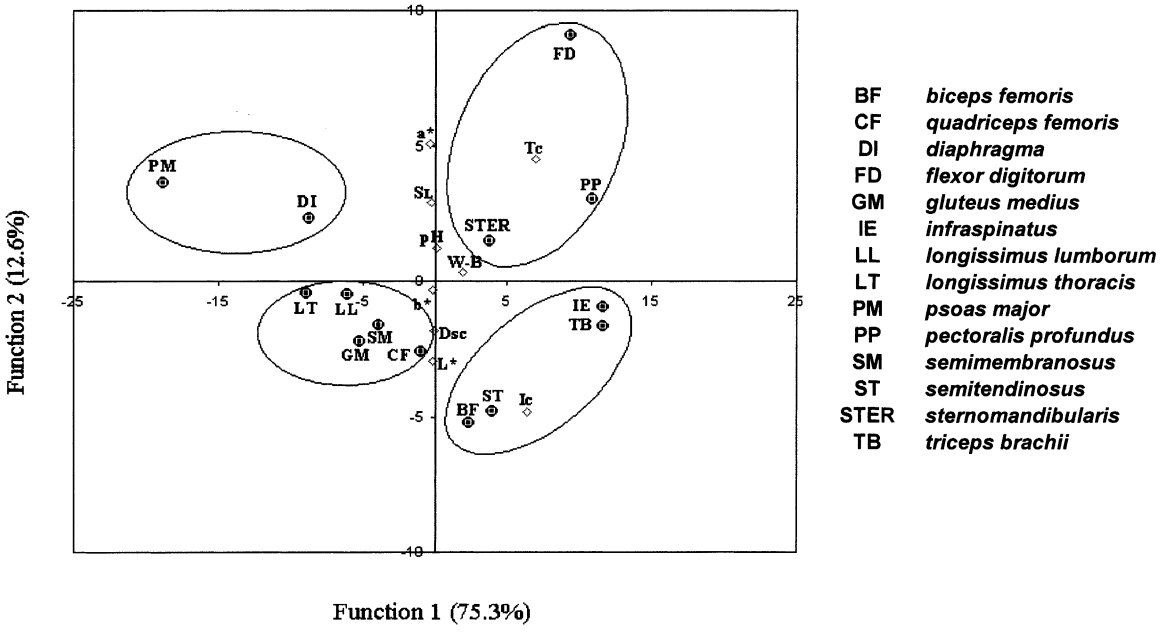

Fig. 1. Scatter plot of the discriminant analysis. W–B (Warner–Bratzler shear force); Tc (total collagen); Ic (insoluble collagen); SL (sarcomere length); DSC (differential scanning calorimetry); L^*, a^*, b^* (colour values).

loin muscles (GM, SM, CF, LT, LL) in a same group, while another group was formed by muscles PM and DI. These results are in agreement with those shown in WBSF analysis; in which DI and PM resulted the most tender muscles (Table 1). Furthermore, Function 1 joined other muscles (PP, STER and FD) classified as the toughest muscles (Table 1) and formed a group (IE, TB, ST and BF) considered as medium toughness muscles. The plot of the Function 1, also related the total and insoluble collagen and WBSF measurements on the right side of the same function with muscle toughness.

As a result of the analysis we achieved a suitable classification of the muscles of the study by means of the physical and chemical parameters or characteristics according to their location in the carcass.

4. Conclusions

Shear values of raw beef muscles (WBSF) were correlated with total and insoluble collagen values for all the muscles analysed. The raw *diaphragm*, considered an inferior muscle in most countries, had low shear values.

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