Heat shock proteins on beef quality

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Abstract – The objective of this work was to quantify heat shock proteins (HSP) 27 and 70 in the *Longissimus dorsi* muscle of cattle during aging and to check their potential as biomarkers for meat quality. A total of 191 steers ½ South African Simmental x ½ Nellore (16–18 months, 391.7±99.7 kg), castrated, and feedlot finished were used. Meat quality was measured by pH, color, cooking loss, and shear force (SF) at 1 and 14 days of aging time. HSP27 and HSP70 were quantified according to the SF values in the more and less tender meat groups, with 20 samples each, for each aging time. HSP27 concentrations in more and less tender meat decrease from 1 to 14 days of aging, and do not differ when evaluated at the same period. HSP70 concentrations in more tender meat increase during aging, and, in less tender meat, there is no difference between periods or at the same period. The correlations between the HSP27 and HSP70 concentrations and meat quality characteristics are low for South African Simmental x Nellore, which indicate the low potential of HSP as biomarkers for these traits, especially for meat tenderness.

Index terms: *Bos taurus indicus*, biomarker, HSP27, HSP70, meat tenderness.

Proteínas de choque térmico na qualidade da carne

Resumo – O objetivo deste trabalho foi quantificar as proteínas de choque térmico (HSP) 27 e 70 no músculo *Longissimus dorsi* bovino, durante a maturação, e verificar seu potencial como biomarcadores na qualidade da carne. Foram utilizados 191 novilhos ½ Simental Sul Africano x ½ Nelore (16–18 meses, 391,7±99,7 kg), castrados e terminados em confinamento. A qualidade da carne foi mensurada por pH, cor, perda de água por cocção e força de cisalhamento (FC) a 1 e 14 dias de maturação. A quantificação de HSP27 e HSP70 foi realizada a partir dos valores de FC, nos grupos de carne mais e menos macia, com 20 amostras cada um, para cada tempo de maturação. As concentrações de HSP27 nas carnes mais e menos macias diminuem no decorrer de 1 a 14 dias de maturação, e não diferiram no mesmo período. As concentrações de HSP70 nas carnes mais macias aumentam com o decorrer da maturação, e, nas menos macias, não há diferença entre períodos ou no mesmo período. As correlações entre as concentrações de HSP27 e HSP70 e as características de qualidade da carne são baixas para Simental Sul Africano x Nelore, o que indica o baixo potencial das HSP como biomarcadores para essas características, especialmente para maciez da carne.

Termos para indexação: *Bos taurus indicus*, biomarcador, HSP27, HSP70, maciez da carne.

Introduction

Tenderness and color are the main attributes associated to beef quality, since they are directly related to consumer satisfaction. Ensuring meat quality regarding palatability, mainly meat tenderness, contributes to more profitable market opportunities for all the meat production chain (Mullins et al., 2016).

Heat shock proteins (HSP) feature anti-apoptotic functions, and studies on meat quality have gained relevance because muscle cells are led to post mortem apoptosis during the process to turn muscle into meat. The HSP protective function slows the muscle-aging rate and attenuates the degradation of myofibrillar proteins (Ouali et al., 2006). HSP are chaperone proteins, which have the capacity to interact with other proteins reversibly and to assist in the formation, folding, and transmembrane transport of these proteins (Castro et al., 2013).

When an organism is subjected to harsh environmental conditions (stress), the metabolism tends to synthesize more HSP, which helps cells to

identify damaged proteins for subsequent repair. In addition, HSP have a crucial role in the maintenance of cellular life, because they inhibit apoptosis (Mullins et al., 2016).

The degradation rate of myofibrillar proteins and consequent impact on tenderness depend on the degradation extent of HSP27 in the meat (Balan et al., 2014), since these proteins are closely related to cytoskeleton actin stability (Boncoraglio et al., 2012). HSP70 has an essential role in the protein repair mechanism in cells subjected to stress. Moreover, it can guide newly synthesized proteins in the folding process (Castro et al., 2013).

There are differences in HSP expression and their relationship with meat quality traits such as tenderness, color, and water-holding capacity (Lomiwes et al., 2014). Researches point to increases in HSP27 in samples of less tender meat (Kim et al., 2008; Pulford et al., 2008, 2009; Carvalho et al., 2014) and in HSP70 in more tender meat (Picard et al., 2010, 2014; Carvalho et al., 2014).

The close relationship between HSP and meat quality traits indicates that these proteins can act as potential quality biomarkers (Ouali et al., 2013; Balan et al., 2014; Cassar-Malek & Picard, 2016). However, it was discovered that HSP expression varies according to the breed, age, sexual condition, and muscles analyzed (Picard et al., 2014; Mullins et al., 2016).

The objective of this work was to quantify HSP27 and HSP70 in the *Longissimus dorsi* muscle of cattle during aging and to check their potential as biomarkers for meat quality.

Materials and Methods

A total of 191 castrated male cattle from crossings between ½ South African Simmental and ½ Nellore, feedlot finished, fed a diet consisting of 56% roughage and 44% concentrate were evaluated. The animals – with an average age of 16–18 months and body weight of 391.7±99.7 kg – were slaughtered in compliance with the technical standards for humane slaughter (Brasil, 2000), in the municipality of Ipuã, in the state of São Paulo, Brazil, between November 2013 and April 2014.

After slaughter, the carcasses were kept under refrigeration at 0–2°C for 48 hours, when temperature and pH were measured with the pH 11 meter (Oakton Instruments, Vernon Hills, IL, USA) in the *Longissimus*

dorsi muscle (LD), between the twelfth and thirteenth ribs. Then, two steaks of 2.54 cm each, from the same part of the LD, were wrapped individually and aged in a cold chamber (0–2°C) for 1 and 14 days. Afterward, the physicochemical analyses were carried out.

The color values were obtained with the CM-2500d portable colorimeter (Konica Minolta, São Paulo, SP, Brazil), from the average of three distinct measurement points, using the luminosity (L*), red/green coordinate (a*), and yellow/blue coordinate (b*) scale (CIE, 1978), with D_{65} light source, 10° observation angle, and cell opening of 30 mm.

Cooking loss (CL) was calculated by the difference in the weight of the samples before and after cooking, expressed in percentage (Koohmaraie, 1996). The steaks were cooked in an electric oven at 170°C until they reached internal temperature of 71°C, which was measured with individual thermocouples connected to the Gulterm 700-10s digital data logger (Gulton Instrumentos de Medição e Automação Indústria e Comércio Ltda., São Paulo, SP, Brazil).

After cooking, six to eight cylinders of 1.27 cm in diameter were removed from each steak by the FG-13B electric punch (Ferrari, Cotia, SP, Brazil). The steaks were sheared using the Salter 235 Warner-Bratzler shear force equipment (Zwick USA, Kennesaw, GA, USA), with an offset probe of 500 mm/min. Shear force (SF) was estimated based on the average measured in the cylinders removed from each sample and expressed in kilograms (AMSA, 1995).

For HSP27 and HSP70 quantification, 40 animals were selected based on the results of pH and tenderness, which were obtained previously. Only animals with final pH below 5.79 were selected. After that, based on the tenderness trait, 20 animals with low SF values (SF ≤ 4.5 kg) and 20 animals with high SF values (SF > 4.5 kg) were chosen, representing animals with more and less tender meat, respectively. Quantification was performed in 40 samples (20 more tender and 20 less tender) at both times of aging (1 and 14 days), totalizing 80 samples.

Samples of 250 µg were homogenized using the T 10 Ultra Turratec disperser (IKA Brasil, Campinas, SP, Brazil) in 2.5 mL extraction solution of phosphate-buffered saline (PBS) 10x concentrated for 15 s at 30,000 rpm. The homogenate was centrifuged at 10,000 x g at 4°C for 30 min, and the supernatant stored at -80°C. The quantifications were performed

using the HSP27 and HSP70 immunoenzymatic kits (MyBioSource.com, San Diego, CA, USA), according to the manufacturer's instructions. All samples were analyzed in duplicate.

Total proteins of the samples were quantified according to Bradford (1976), and the results of the quantification of HSP27 and HSP70 were expressed based on the total protein from each sample (pg HSP/mg total protein).

To assess the HSP27 and HSP70 concentrations and indicator variables of meat quality, a mixed linear model was used, including fixed effects of meat tenderness (less tender x more tender), aging time (1 day x 14 days post mortem), and the interaction between meat tenderness and aging time, in addition to random, animal, and residue effects. Repeated measures were considered in the same experimental units, adopting an unstructured matrix for the covariance evaluation between measurements. The analyses were carried out using the PROC Mixed procedure of the SAS software, version 9.1.4 (SAS Institute Inc., Cary, NC, USA). To assess the possible relationship between the HSP concentrations and the indicators of meat quality, Pearson product-moment correlation analyses were performed with the PROC CORR procedure also of SAS.

Results and Discussion

The average values of pH (Table 1) indicate that the conversion process of muscle into meat occurred slowly and was suitable during the 48 hours post mortem, generating meat with normal pH. The reduction in pH during the post mortem process is a result of muscle effort to maintain constant the levels of adenosine triphosphate (ATP). Therefore, the available energy reserves in the form of muscle glycogen begin to be metabolized via anaerobic glycolysis, forming lactate, and their accumulation in the tissue favors a gradual decrease in intramuscular pH (Ylä-Ajos et al., 2006).

The average CL values (Table 1) were considered normal and similar to those found in other studies, in which the average values were 21.06 and 29.96% in bovine samples (*Bos indicus* x *B. taurus*) at 0 and 14 days of aging, respectively (Aroeira et al., 2016). During the cooking process, protein denaturation occurs due to heat, which changes the interfibrillar spaces and causes the shortening of muscle fiber sarcomeres. Consequently, there is a decrease in the water holding capacity of meat and a consequent release

of exudates (Huff-Lonergan & Lonergan, 2005). This is an indicative that the greater denaturation of myofibrillar and sarcoplasmic proteins at 14 days of aging may have affected the increase in the average CL value observed here.

Regarding color characteristics, a study on Simmental animals (Soji & Muchenje, 2016) showed average values of L* (31.1), a* (14.5), and b* (10.8) similar to those obtained in this study (Table 1). Frylinck et al. (2013) and Chambaz et al. (2003) also reported similar values for L* and a*, but different ones for b* (6.3 and 4.1, respectively). It should be noted that meat color varies due to several biochemical changes in myoglobin. According to Karamucki et al. (2011), low b* values are characterized by a higher deoxyglobin concentration (reduced myoglobin) in the sample, which results in a red-purple color. A higher concentration of this compound in meat can be justified by the vacuum packaging of samples. In this type of packaging, there is metmyoglobin formation, which is oxidized myoglobin (brown color). With the absence

Table 1. Number of observations (No.), mean, standard error (SE), and maximum and minimum values for quality traits of *Longissimus dorsi* beef from Simmental South African x Nellore cattle.

Quality trait[1]	No.	Mean	SE	Minimum	Maximum
pH 1 at day	23	5.4665	0.1076	5.2100	5.6500
pH 14 at days	33	5.5982	0.1221	5.2900	5.8400
CL 1 at day	40	27.6031	4.7276	17.8526	42.2171
CL 14 at days	39	28.1451	3.1992	22.0033	36.0754
L* 1 at day	40	31.5968	5.0644	19.9100	38.5000
L* 14 at days	39	30.7351	5.4986	19.4500	43.3400
a* 1 at day	40	14.5733	3.5459	8.9600	24.1500
a* 14 at days	39	16.2846	2.9567	10.6100	21.5000
b* 1 at day	40	9.7420	4.2376	4.6900	21.0500
b* 14 at days	39	15.6636	4.9071	6.9500	22.7400
SF 1 at day	40	8.5875	1.4805	4.0500	11.2500
SF 14 at days	39	4.7436	1.8437	1.9500	9.7500

[1]pH at 1 and 14 days, intramuscular pH measured at 1 and 14 days of aging, respectively; CL, at 1 and 14 days, cooking loss (%) measured on steaks at 1 and 14 days of aging; L*, a*, and b* at 1 and 14 days, color measurements performed on steaks at 1 and 14 days of aging; and SF at 1 and 14 days, shear force (kg) determined on steaks at 1 and 14 days of aging.

of oxygen in the environment, the metmyoglobin pigment is reduced by the normal reducing conditions of meat, forming deoxyglobin.

In an experiment with South African x Simmental crossbreed, feedlot finished, Frylinck et al. (2009) observed average SF values of 9.52 and 6.22 kg, at 1 and 14 days of aging, respectively. The variation of approximately 35% in SF values confirms the findings of the present study, in which a variation of 45% was verified between 1 and 14 days of aging. This improvement in tenderness values (SF reduction) is due to the proteolysis of myofibrillar proteins, which stand out during the meat aging process (Koohmaraie, 1996).

When evaluating Simmental animals, Soji & Muchenje (2016) obtained mean SF values of 4 kg in mature samples after 7 days. This difference in tenderness compared with the values found in the present study can be explained by the interference of the *B. taurus indicus* genotype in the crossing, since zebu animals tend to present less tender meat (Whipple et al., 1990; Restle et al., 1999). Whipple et al. (1990) pointed out that 37.5% of zebu blood in the crossbreeds is enough to interfere in meat tenderness, resulting in less tender samples.

The average HSP27 concentration decreased during the aging period (Table 2). According to Pulford et al. (2008), when the environment reaches the isoelectric point (IP) of a protein, a lack of net load reduces its solubility and causes its precipitation, eliminating its effect on the post mortem process of the muscle. The IP of HSP70 is 5.5, which is very close to the meat final pH observed in the present work, suggesting that these proteins were precipitated (inactive) in the samples. HSP27 has higher IP, around 5.98, which allows it to keep its activities longer.

HSP27 concentrations in more and less tender meat decreased significantly (p < 0.05) during aging time.

When concentrations were compared at the same aging time, i.e., both at 1 and 14 days, there was no significant difference between the more and less tender meat samples (Table 3).

The assessment of the average HSP70 concentrations in more tender samples showed significant difference (p < 0.05) between aging times, with an increase in protein concentration from 1 to 14 days. No significant difference was observed in less tender samples, or in more and less tender meat compared at the same aging period (Table 3).

Mullins et al. (2016) reported that HSP27, αβ-crystalline, and HSP70 work together for the maintenance of cellular life after a stress situation, preventing apoptosis. HSP27 and αβ-crystalline play a protection role against the degradation and denaturation of myofibrillar proteins, releasing damaged proteins to HSP70, which will repair and renovate its protein biological activity (Wang & Spector, 2001). Therefore, a similar behavior was expected for the HSP27 and HSP70 concentrations. However, in samples of tender

Table 2. Number of observations (No.), mean, standard error (SE), and maximum and minimum values of heat shock proteins 27 (HSP27) and 70 (HSP70) of *Longissimus dorsi* beef from Simmental South African x Nellore cattle.

Characteristic[1]	No.	Mean	SE	Minimum	Maximum
HSP27 at 1 day	40	192.22	42.626	140.25	377.27
HSP27 at 14 days	40	153.26	50.721	77.352	256.39
HSP70 at 1 day	40	23.562	8.531	11.703	45.366
HSP70 at 14 days	40	29.693	7.9502	14.489	54.078

[1]HSP27 at 1 and 14 days, concentration of HSP27 [(pg / mg) / total protein] in the samples at 1 and 14 days of aging, respectively; and HSP70 at 1 and 14 days, concentration of HSP70 [(pg / mg) / total protein] in the samples at 1 and 14 days of aging.

Table 3. Mean and standard error (SE) estimates of the concentrations of heat shock proteins 27 (HSP27) and 70 (HSP70), at 1 and 14 days of aging, regarding the tenderness of *Longissimus dorsi* beef from Simmental South African x Nellore cattle[1].

Meat tenderness	HSP27				HSP70			
	1 day		14 days		1 day		14 days	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
More tender	185.54Aa	9.5339	156.76Ba	11.4617	21.3995Ba	1.8678	31.5468Aa	1.7501
Less tender	198.9Aa	9.5339	149.76Ba	11.4617	25.7237Aa	1.8678	27.8401Aa	1.7501

[1]Means followed by equal letters, uppercase in the rows and lowercase in the columns, do not differ by Fisher's t-test, at 5% probability.

meat, the HSP70 concentrations increased along aging time.

The reduction in HSP27 and HSP70 can positively interfere in meat tenderness and result in more tender samples (Kim et al., 2008; Carvalho et al., 2014). However, the data obtained in the present study showed that the HSP27 concentration in the samples did not interfere in meat tenderness, as a similar decrease was observed in both groups analyzed (more and less tender meat) during aging. Therefore, HSP27 could not be considered a biomarker for meat tenderness of South African Simmental x Nellore animals.

HSP70 may positively interfere in the meat tenderization process, since its concentration increased during the aging period in the more tender group. These results are the inverse of those reported in the literature (Kim et al., 2008; Carvalho et al., 2014), whose data showed that the decrease in HSP70 interfered in the meat tenderization process. Therefore, the results presented here have led to new questions about HSP70 behavior and its interference in meat tenderness. Further studies should be performed to confirm the effects of HSP70 on crossbreed animals.

There was a significant correlation ($p < 0.05$) between HSP27 and b* values at 14 days, and between HSP70 and L* values also at 14 days, but these correlations were low. For the other traits analyzed, there was no significant correlation (Table 4).

HSP27 has been correlated with color attributes, with a greater expression of this protein in darker samples (Joseph et al., 2012). However, the data obtained in the present study indicated an opposite result, in which animals with lower HSP27 concentrations tended to have meat with higher b* values (darker). Meat color changes due to the molecular behavior of myoglobin, which is considered unstable, because it turns into oxymyoglobin in the presence of oxygen and into metmyoglobin in the absence of it. Oxymyoglobin is responsible for generating redder meat, while metmyoglobin generates browner meat. Gagaoua et al. (2017) suggested that, during the post mortem process, HSP27 activity might affect myoglobin stability by avoiding protein aggregation and denaturation. However, the specific role of HSP27 in meat color stability has not yet been fully understood.

The increase in HSP70 expression generates meat with lower L* values, that is, less bright and less pale. This change in meat brightness can be attributed to a cascade of reactions that occur during the post mortem process, where HSP70 protects structural proteins against proteolytic degradation and the consequent structural change of meat pigmentation (Gagaoua et al., 2015).

HSP27 presented a correlation trend for some meat quality traits, such as CL at 14 days, L* at 14 days, and SF at 1 day ($F < 0.1$); once again, these correlations were considered low. Previous studies indicated that both HSP27 and HSP70 have positive correlations with meat tenderness (Kim et al., 2008; Carvalho et al., 2014). This difference between the data observed here and in the literature can be explained by the different muscles, animal age, sex, and subspecies analyzed, resulting in varying HSP concentrations. Zebu breeds have shown higher concentrations of HSP27 and HSP70 than taurine ones, for example (Picard et al., 2014; Mullins et al., 2016). However, studies on HSP behavior in crossbreed animals (*B. taurus taurus* x *B. taurus indicus*) and its correlation with meat quality traits are still scarce.

This work showed a differentiated effect of HSP27 and HSP70 on meat quality traits, with possible changes in the protein metabolism of crossbreed animals.

Table 4. Pearson moment-product correlation coefficients between the concentrations of heat shock proteins 27 (HSP27) and 70 (HSP70) and the meat quality traits of *Longissimus dorsi* beef from Simmental South African x Nellore cattle.

Quality trait[1]	HSP27	P-value	HSP70	P-value
pH 1 at day	-0.03851	0.8615	0.09480	0.6670
pH at 14 days	-0.16522	0.3582	0.02677	0.8824
CL at 1 day	-0.14000	0.3889	-0.16556	0.3073
CL at 14 days	-0.28204	0.0819	-0.03572	0.8291
L* at 1 day	0.20596	0.2023	0.08637	0.5962
L* at 14 days	0.30864	0.0559	-0.40734	0.0101
a* at 1 day	-0.11794	0.4686	-0.26349	0.1004
a* at 14 days	-0.11508	0.4854	0.19677	0.2299
b* at 1 day	-0.05743	0.7249	-0.07286	0.6550
b* at 14 days	-0.36626	0.0218	0.16327	0.3207
SF at 1 day	-0.26876	0.0936	0.03245	0.8425
SF at 14 days	-0.12169	0.4605	-0.07711	0.6408

[1]pH at 1 and 14 days, intramuscular pH measured at 1 and 14 days of aging, respectively; CL at 1 and 14 days, cooking loss (%) measured on steaks at 1 and 14 days of aging; L*, a*, and b* at 1 and 14 days, color measurements performed on steaks at 1 and 14 days of aging; and SF at 1 and 14 days, shear force (kg) determined on steaks at 1 and 14 days of aging.

Conclusions

1. HSP27 concentrations in more and less tender meat decrease from 1 and 14 days of aging, and do not differ when compared at the same aging period.

2. HSP70 concentrations in more tender meat increase from 1 to 14 days of aging, and do not differ in less tender samples and at the same aging period.

3. The correlations between the HSP27 and HSP70 concentrations and meat quality traits are low for South African Simmental x Nellore cattle, which indicate their low potential as biomarkers for these traits, especially for meat tenderness.

Acknowledgments

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