

Effect of high-pressure processing on texture and color of crossbreed F1 Senepol/Nelore cattle beef

Otávio Cabral Neto¹, Rosires Deliza², Sabrina Luzia Grégio de Sousa³, Tatiana Saldanha⁴, Ormindo Domingues Gamallo⁴, Wellington Barros dos Santos^{5*}, Amauri Rosenthal²

¹Instituto Federal de Educação, Ciência e Tecnologia do Tocantins, Av. LO 5 – 77021-090 – Palmas, TO – Brasil.

²Embrapa Agroindústria de Alimentos, Av. das Américas, 29501 – 23020-470 – Rio de Janeiro, RJ – Brasil.

³Universidade Federal Rural do Rio de Janeiro – Depto. de Produção Animal, BR 465, km 07 – 23890-000 – Seropédica, RJ – Brasil.

⁴Universidade Federal Rural do Rio de Janeiro – Depto. de Tecnologia de Alimentos, BR 465, km 07 – 23890-000 – Seropédica, RJ – Brasil.

⁵Universidade Federal de Lavras/Instituto de Ciências Naturais – Depto. de Biologia, Av. Bueno da Fonseca, 543 – 37200-000 – Lavras, MG – Brasil.

*Corresponding author <wellpsibarro@gmail.com>

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ABSTRACT: This research study endeavored to examine the impact of high-pressure processing (HPP) on the texture and color of post-rigor mortis beef, explicitly focusing on the Longissimus dorsi muscle obtained from the crossbred F1 Senepol/Nelore breed of cattle. Pressure levels ranging from 100 to 400 MPa were exerted at varying processing times on beef sourced from animals of both genders (males and females). Subsequently, the samples were assessed for tenderness, cooking loss, and color. HPP at 100 and 200 MPa promoted significant increases ($p < 0.05$) in tenderness regardless of sex, based on decreases in shear force in instrumental texture analyses. A significant increase in cooking loss was detected at higher pressures (more than 300 MPa). HPP made a statistically significant impact ($p < 0.05$) on specific color parameters, and overall, the treatment at 200 MPa resulted in more positive effects.

Keywords: *Longissimus dorsi*, cooking loss, feedlot, instrumental texture analyses, tenderness

Brazil has the world's largest cattle herd, with over 234 million head (IBGE, 2022). Approximately 80 % of this herd consists of zebu breeds (*Bos taurus indicus* L. 1758), such as Nelore, known for their hardiness but lower meat tenderness. Additionally, Brazil has European-adapted breeds like Senepol (*Bos taurus taurus* L. 1758). To enhance production efficiency and meat quality, techniques like crossbreeding Zebu cattle with European breeds have been investigated. This strategy enhances carcass formation and meat tenderness through heterosis (Oliveira, 2018).

High-pressure processing (HPP) is used in meat, dairy, vegetable, and beverage production. However, its effects on post-rigor mortis beef from crossbred zebu cattle are poorly understood. The global HPP food market was valued at US\$ 9.8 billion in 2015 and is projected to reach US\$ 54.77 billion by 2025 (Huang et al., 2017; Bonfim et al., 2019).

Tenderness is the primary quality influencing meat consumption, with consumers willing to pay premium prices for tender meat (Hope-Jones et al., 2010). HPP has shown potential for improving meat tenderness quickly and at lower temperatures, with minimal changes to other characteristics (Bajovic et al., 2012; Abera and Yildiz, 2019).

This study's objective was to evaluate the impact of HPP on tenderness, cooking loss, and color in fresh meat from F1 Nelore/Senepol crossbred cattle. Given the scarcity of studies in this area, particularly in Brazil where

the methodology differs from that applied internationally (Bolumar et al., 2013), this research aims to identify the best processing conditions for Brazilian beef.

The animals were bred in Uberaba, located at 19°44'54" S, 47°55'55" W, altitude 830 m, in the Minas Gerais state, Brazil. This study employed ten animals of the F1 Nelore/Senepol breed, representing a cross between *B. taurus indicus* and *B. taurus taurus* cattle, with an equal distribution of five males (uncastrated) and five females. No maturation time was allowed between the application of the HPP and the analyses. After processing, the meats were immediately frozen and thawed only one day before analysis.

The animals were fed in the field and were subjected to 120 days of confinement before being slaughtered at 21 months of age, classified as "d" concerning milk teeth dentition. After a 24-h fast, they had average weights of 553.40 ± 34.83 kg for males and 500.60 ± 21.16 kg for females. Slaughter and carcass processing took place at the Real Slaughterhouse in Uberaba, resulting in average weights of 313.48 ± 7.30 kg for males and 269.80 ± 9.10 kg for females, with yields of 57 and 54 %, respectively. The carcasses were classified according to the degree of finish: males as grade four, with a uniform fat layer of 7.3 mm, and females as grade three, with a moderate fat thickness of 4.7 mm (MAPA, 2004).

The hindquarters were stored at 3 °C and cooled to 10 °C within 24 h. The pH was monitored during

cooling, reaching 5.8 after 24 h, indicating the end of *rigor mortis*. The water activity in untreated meat samples was measured using Aqualab 4TE equipment (Decagon Devices), with an average value of 0.99. After *rigor mortis*, the meat was deboned and cut in rooms maintained at 10 °C. This study focused on the sirloin (*Longissimus dorsi*) between the ninth and twelfth ribs. The sirloin sections were sliced into 2.5 cm pieces, vacuum-packed in polyethylene bags, and stored at 5 °C. Packaging equipment (Selovac 200B) with a vacuum set to 20 (50 Pa) was used (Cabral Neto et al., 2015).

The experiment was conducted on meat from both male and female animals, with slices of striploin subjected to different pressures (100, 200, 300, and 400 MPa) and durations (T1: the time required to reach the designated pressure; and T2: T1 plus an additional 15 min, with T2 equating to a traditional high-pressure process), with immediate decompression following each pressure application. Both pressurization conditions were executed using hydrostatic pressure processing equipment (Stansted Fluid Power, model S-FL-850-9-W).

The samples were placed in a perforated cylindrical holder (4 cm in diameter, 30 cm in length, 377 mL total volume, 345 mL usable) to facilitate the circulation of the pressurization liquid, 70 % ethanol. This liquid circulated inside the vessel and around the samples during pressurization. The holder was then inserted into a stainless-steel pressure vessel and hermetically sealed.

The pressurization system was engaged in two stages: first, a vacuum pump established a pre-load and sealed the container; second, a hydraulic pump raised the pressure to the desired level at a constant rate of 7 MPa s⁻¹. The initial temperature of the samples was 5 °C to control adiabatic heating, ensuring a maximum exit temperature of 18 °C at 400 MPa to prevent undesirable cooking. After each cycle, the samples were frozen and stored for analysis. The control sample, which was not subjected to high pressure, was cooled to 15 °C for a period of 24 h and then frozen using air freezing (convection). The eight pressurized samples were stored at -20 °C for five days before analysis. Before conducting the analysis, all samples, including the control ones, were thawed at 6 °C for 24 h the preceding day.

Steaks (2.5 cm thick) were cooked on a Vicini electric grill (Model 110V EPV-853) with wavy heating plates on both sides, preheated to 170 °C. Each steak was weighed before cooking, and the grill lid was closed during cooking. The internal temperature was monitored with a metal probe thermometer until it reached 72 °C; then, the steaks were weighed again. Cooking loss was calculated as the weight difference before and after cooking. The samples were labeled, sealed in polyethylene bags, and stored at 4 °C for 24 h, following AMSA (1995).

Six samples with a diameter of 1.25 cm were taken from the grilled steaks in a direction parallel to the longitudinal orientation of the muscle fibers. A TA-HDi texture analyzer (Texture Technologies Corp./Stable

Micro Systems) with a 1 mm thick Warner-Bratzler blade was used to determine the shear force. The equipment was calibrated using a traceable standard weight of 50 kg. The blade's ascent and descent speeds were consistently set to 200 mm min⁻¹ (AMSA, 1995). The blade was positioned at a distance of 25 mm from the platform. Each sample was submitted to a single cut, and the results were expressed in Newtons (N). The test was conducted in six replicates for each sample to ensure accuracy and consistency.

For the color analysis of fresh beef samples, Color Quest XE equipment from Hunter Lab was used, employing the CIE L*a*b* color scales following the methodology of Papadakis et al. (2000). Meat samples were reoxygenated for 30 min before color analysis on an instrument with an opening of 25 mm in diameter, illuminant D65, and observer 10° angle. L* is the luminance or lightness component, which ranges from 0 (black) to 100 (white). Six replicates were selected for each sample to ensure data reliability and robustness. The experimental design followed a completely randomized approach, and the data was submitted to analysis of variance (ANOVA), Tukey's test, and F-test at a significance level of 5 %. XLSTAT software was used for the ANOVA and mean comparisons.

The results of shear force, which is indicative of tenderness, and cooking loss are presented in Table 1. Non-pressurized female meat was significantly ($p < 0.05$) more tender than males based on shear force values. Significant reductions ($p < 0.05$) were recorded for shear force in samples pressurized at 100 and 200 MPa regardless of sex, enhancing tenderness of 38 % for males and 31 % for females in samples pressurized at 200 MPa. Pressures above 200 MPa reduced tenderness compared to non-pressurized samples, regardless of sex.

The results indicate that there was no statistically significant difference ($p > 0.05$) in cooking loss between the non-pressurized meat and the samples pressurized at 100 and 200 MPa in condition T1, regardless of sex (Table 1). However, cooking loss was significantly increased ($p < 0.05$) when processed at any pressure level at T2. An increase in pressurization time was associated with a higher cooking loss ($p < 0.05$), indicating a relationship between pressure level and pressurization time.

High-pressure processing resulted in a significant effect ($p < 0.05$) on certain color parameters (L*, a* and/or b*) of *Longissimus dorsi* (Table 2), which can be better visualized in Figure 1. Compared to nonpressurized samples, the meat of whole males and females showed no significant changes. A significant increase ($p < 0.05$) in L* resulted in pressurized whole male meat above 200 MPa and for females at 100 MPa and up for T1. Male samples pressurized at T2 presented significantly enhanced L* ($p < 0.05$) above 200 MPa. As regards a* gender, this did not show any influence on T1. However, HPP reduced in values as the pressure rose, leading to significant differences ($p < 0.5$) for males above 200 MPa and females over 100 MPa.

Table 1 – Shear force and cooking loss, including mean and standard deviation values, were assessed in F1 Senepol/Nelore beef (*Longissimus dorsi*) treated with high hydrostatic pressure, and the results were compared to untreated meat.

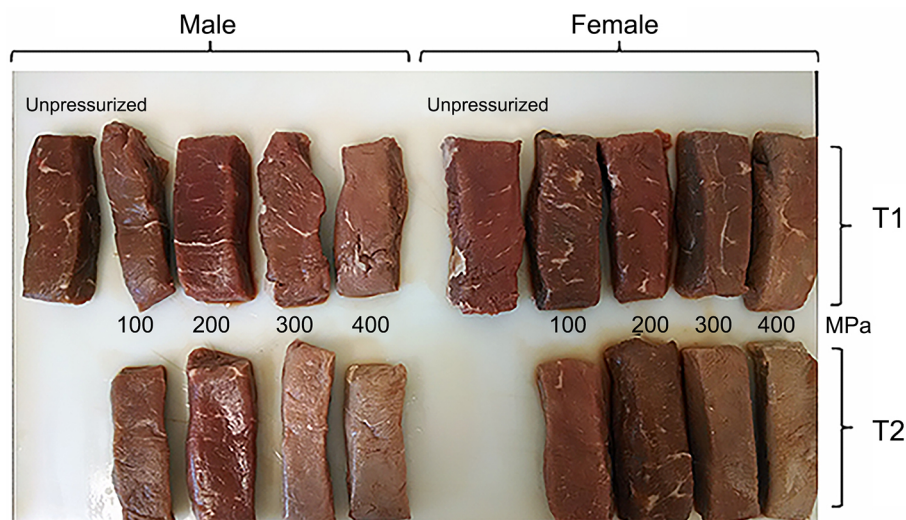
Treatment time	Pressure	Shear force		Tenderness increasing ¹		Cooking loss	
		Male	Female	Male	Female	Male	Female
	MPa	N				%	
T1	Unpressurized	32.20 ± 0.80 ^a	28.11 ± 1.88 ^d			19.96 ± 1.24 ^a	19.27 ± 2.43 ^a
	100	22.56 ± 1.66 ^b	22.49 ± 1.38 ^b	29.94	19.99	19.89 ± 2.06 ^a	20.04 ± 1.76 ^a
	200	19.86 ± 2.57 ^a	19.26 ± 1.72 ^a	38.32	31.48	20.68 ± 2.24 ^a	20.91 ± 1.24 ^a
	300	35.37 ± 1.72 ^f	32.77 ± 1.93 ^e	−09.85	−16.57	27.76 ± 1.15 ^{bc}	28.27 ± 0.96 ^{bc}
	400	44.23 ± 2.46 ^{hi}	42.26 ± 0.92 ^h	−37.36	−50.33	30.51 ± 2.75 ^d	29.58 ± 1.75 ^{cd}
T2	100	24.47 ± 0.85 ^{bc}	26.07 ± 1.72 ^{cd}	24.01	07.26	25.15 ± 1.56 ^b	25.63 ± 2.25 ^b
	200	23.03 ± 1.06 ^b	23.34 ± 1.59 ^b	28.48	16.97	28.56 ± 1.70 ^{bc}	26.59 ± 1.55 ^b
	300	39.24 ± 1.65 ^g	36.79 ± 1.89 ^f	−21.86	−30.88	35.55 ± 1.37 ^{ef}	33.09 ± 1.27 ^e
	400	49.08 ± 0.79 ^j	45.09 ± 1.08 ⁱ	−52.42	−60.41	37.53 ± 0.69 ^f	34.59 ± 0.98 ^e

Different letters within the same column denote a statistically significant difference ($p < 0.05$) as determined by the Tukey's test. ¹Negative value indicate a reduction in tenderness. N = newton; T1 = the duration required to reach the predetermined pressure, followed by immediate pressure release; T2 = T1 extended by an additional 15 min.

Table 2 – Color parameters, including mean and standard deviation values, of F1 Senepol/Nelore beef (*Longissimus dorsi*) subjected to high hydrostatic pressure treatment were compared with those of untreated meat.

Treatment time	Pressure	Factor of color					
		L*		a*		b*	
		Male	Female	Male	Female	Male	Female
	MPa						
T1	Unpressurized	34.09 ± 0.69 ^{ab}	33.76 ± 3.27 ^{ab}	21.74 ± 2.04 ^{abc}	18.40 ± 3.60 ^{cde}	12.67 ± 2.13 ^{abc}	11.01 ± 2.20 ^{bcd}
	100	34.95 ± 2.00 ^{abc}	34.76 ± 3.33 ^{abc}	21.58 ± 2.24 ^{abcd}	17.71 ± 3.96 ^{def}	14.11 ± 4.34 ^a	10.23 ± 1.25 ^{cde}
	200	33.02 ± 2.27 ^a	37.95 ± 2.46 ^{bcd}	19.24 ± 1.61 ^{bcd}	16.62 ± 3.87 ^{ef}	12.86 ± 3.41 ^{abc}	9.13 ± 2.48 ^{def}
	300	39.10 ± 1.42 ^{cde}	39.94 ± 2.39 ^{de}	17.44 ± 1.45 ^{ef}	16.61 ± 2.98 ^{ef}	11.89 ± 1.31 ^{abcd}	9.23 ± 2.48 ^{def}
	400	43.70 ± 1.70 ^{ef}	43.32 ± 1.35 ^{fg}	15.79 ± 1.16 ^{ef}	16.82 ± 4.67 ^{ef}	11.55 ± 0.86 ^{abcd}	7.84 ± 2.30 ^{efg}
T2	100	33.04 ± 2.84 ^a	36.85 ± 1.38 ^{abcd}	25.10 ± 1.35 ^a	16.94 ± 0.34 ^{ef}	13.85 ± 1.64 ^{ab}	10.20 ± 1.98 ^{cde}
	200	38.40 ± 1.43 ^{bcd}	35.46 ± 3.07 ^{abcd}	24.10 ± 0.89 ^a	14.15 ± 1.85 ^f	10.95 ± 1.51 ^{bcd}	7.39 ± 1.16 ^{efg}
	300	50.51 ± 1.94 ^{gh}	52.21 ± 1.29 ^{hi}	23.00 ± 1.01 ^{ab}	13.47 ± 3.87 ^f	10.08 ± 1.85 ^{cde}	7.47 ± 1.12 ^{efg}
	400	51.66 ± 1.77 ^{hi}	56.44 ± 2.47 ⁱ	18.18 ± 1.57 ^{cde}	13.99 ± 4.38 ^f	6.53 ± 0.89 ^g	5.03 ± 0.23 ^g

Distinguishing letters within the same factor indicates a significant difference ($p < 0.05$) as determined by the Tukey's test. L* = luminosity (0 = black and 100 = white); a* = intensity of green/red (−80 to 0 = green, from 0 to +100 = red); b* = intensity of blue/yellow (−100 to 0 = blue, from 0 to +70 = yellow). Distinct letters in the same column denote a significant difference ($p < 0.05$) according to the F-test. T1 = the time needed to attain the pre-set pressure, immediately followed by pressure release; T2 = T1 extended by an additional 15 min.

**Figure 1** – Visual assessment of color in *Longissimus dorsi* meat samples from male and female crossbred F1 Nelore/Senepol cattle subjected to high hydrostatic pressure treatment. T1 = the duration required to reach the predetermined pressure, followed by immediate pressure release; T2 = T1 extended by an additional 15 min.

Female cattle produce more tender meat than castrated males, who produce more tender meat than intact males (Luchiari Filho, 2000). This difference is due to the testosterone in intact males, which increases weight gain, feeding efficiency, and muscle fibers (Lawrie, 1985; Luchiari Filho, 2000). Testosterone also boosts calpastatin activity and reduces protein degradation and muscle tenderness by inhibiting calpain, an enzyme crucial to meat tenderness (Hedrick, 1994; Luchiari Filho, 2000). Despite these differences, the meat from both sexes in our study was considered tender, with shear force below 45 N, or even highly tender (< 36 N) (Knapp et al. 1989; Shackelford et al., 1991).

Compared to our previous study on Nelore animals finished on pasture (Cabral Neto et al., 2015), we found similar shear force values between Nelore and crossbred F1 Senepol/Nelore cattle. Despite the slaughter age being six months younger for the crossbreeds, they had higher carcass weight (around 83 kg) and better fat distribution. This suggests that crossbreeds' high zebuine blood level suggests that the high zebuine blood level in crossbreeds did not allow Senepol to directly contribute to tenderness. However, Senepol, well adapted to Brazilian conditions, resulted in larger meat cuts with better fat distribution, leading to more tender meat due to low collagen crosslinking.

Breeding *B. taurus* and *B. indicus* leverages the complementary qualities and benefits of heterosis (Gama, 2002). The lower shear force values observed in the meat of F1 Senepol/Nelore cattle compared to other studies with zebu and crossbred animals (Crouse et al., 1989; Johnson et al., 1990) may result from reduced slaughter age, genetic selection in the Nelore breed (maternal line), and the use of Senepol (*B. taurus taurus*) for breeding and finishing under confinement.

The calpain level decreased with meat pressurization above 100 MPa, being highly reduced at 300 MPa (Homma et al., 1995, 1996). Additionally, while calpain partially resisted up to 200 MPa, calpastatin was inactivated at 100 MPa. Furthermore, HPP promoted the liberation of liposominal cathepsins, which increased their activity in the muscle, and liberated Ca^{+2} from sarcoplasmic reticulum thus activating calpain. Consequently, the elevation of calpain and cathepsin activities in pressurized meat, and inactivation of cathepsin verified at 200 MPa resulted in meat tenderization (Cabral Neto et al., 2011).

Other studies have found similar improvements in meat tenderness with HPP at 200 MPa. However, pressures as high as 400 MPa can decrease tenderness (Ma and Ledward, 2004; Sikes et al., 2010; Sun and Holley, 2010). Bowker et al. (2007, 2008) and Solomon et al. (2006) reported significant reductions in shear force for Brahman (*B. indicus*) meat subjected to high hydrodynamic pressure (HDP), showing reductions of 29 and 23 %, respectively. Although HDP, rather than HPP, was used to generate pressure impulses, the

processes are comparable when a single, brief pulse is applied, as in T1 of the present study. This differs from maintaining pressure for a specific duration, as in T2. Thus, T1 can be seen as a hydrodynamic pressure process which generates a single pulse using the traditional HPP method, unlike the original HDP method used by Solomon et al. (2006).

High-pressure processing is efficient in tenderizing post-rigor zebu meat at 200 MPa, having been observed in fiber conformation, calpain activation and increased cathepsin activity due to the release of Ca^{2+} from the sarcoplasmic reticulum (Cabral Neto et al., 2015). Both sexes achieved shear force values below 20 N, classifying the meat as highly tender. These results, along with improved cut size and quality, suggest potential for this product in the specialty meat market, adding value. This is significant for Brazilian meat, as 80-90 % of slaughtered cattle are intact males, which typically have lower fat and tenderness.

As the effect of processing time (T1 and T2) at the same pressure level, it was possible to see that the increase in time (T2) resulted in the same effect as that observed for shorter treatment (T1), although with lower effectiveness in reducing the shear force for pressures up to 200 MPa, similar to that observed in our previous study with the Nelore (Cabral Neto et al., 2015).

These results differ from those obtained by Ma and Ledward (2013) and Buckow et al. (2013), who maintained that the benefits of HPP processing on *post-rigor* meat are not evident or can only be relevant when processing at higher temperatures. Our results, in the same way as Cabral Neto et al. (2015), showed that in addition to being effective for improving the tenderness of zebuine meat, the processing at pressures below 300 MPa can be used in combination with other technologies for animal production (nutrition, genetics, crossbreeding, etc.) for obtaining meat of higher quality, fulfilling demands of specific markets.

Cabral Neto et al. (2015) obtained similar results for zebuine meat at *post rigor* stage. Some authors described the reduction in water binding capacity at high pressure levels (Crehan et al., 2000; Jung et al., 2000). Jung et al. (2000) proposed that the decrease in the meat's water retention capacity can be attributed to the significant myofibrillar contraction and alterations in protein structure that occur when subjected to high pressure levels. Additionally, sarcoplasmic proteins, which are known to play a crucial role in the water-binding properties of meat muscle (Joo et al., 1999), may also be influenced during the cooking process, thereby contributing to a reduction in water retention capacity.

In a study conducted by Marcos et al. (2010), negative correlation was observed between the solubility of sarcoplasmic proteins and the moisture content of bovine muscle (specifically *Longissimus dorsi*). This finding suggests that the denaturation

of sarcoplasmic proteins induced by pressurization may have a detrimental effect on water binding in pressurized meat. In other words, when sarcoplasmic proteins denature due to the pressure treatment, it may lead to reduced water retention in the meat, as these proteins play a role in binding water within the muscle tissue.

Studies by McArdle et al. (2010), and Ha et al. (2017) found that meat subjected to 300 and 400 MPa pressure exhibited higher cooking loss than 100 and 200 MPa treatments. Significant differences ($p < 0.05$) were observed at 400 MPa, indicating a greater cooking loss than at lower pressures. Additionally, higher pressures (300 and 400 MPa) resulted in significantly higher cooking loss ($p < 0.01$) compared to 200 MPa, suggesting a negative impact on the meat's water-binding properties.

No significant differences were found in unpressurized samples between meat from intact males and females, possibly because of slaughtering age affecting myoglobin concentration and lower antioxidant enzyme activities, which tends to increase with age (Cho et al., 2015). Fernandes et al. (2008) similarly found no significant differences in color parameters (L^* , a^* , b^*) between castrated males, intact males, and females.

Growth in L^* (whitening) with the elevation of pressure mainly above 200 MPa were reported by several authors (McArdle et al., 2010; Marcos et al., 2010; Bajovic et al., 2012; Marcos and Mullen, 2014; Cabral Neto et al., 2015; Guillou et al., 2017; Bak et al., 2019). However, the acceptable value for L^* in bovine meat would be 24 to 39, which in our case would include all the pressurized meat up to 300 MPa at T1 Purchas (1988).

Consistent with Purchas (1988), meat processed at pressures up to 200 MPa is considered acceptable in terms of L^* , regardless of sex. Increasing pressurization time did not improve color, with T1 being more suitable for processing than T2, benefiting industrial production with higher output and lower costs. Pressures of 300 and 400 MPa significantly increased the L^* value ($p < 0.05$), with the whitening effect attributed to protein coagulation, globin protein denaturation, and the displacement or removal of heme groups, as noted in studies by Carlez et al. (1995) and Guillou et al. (2017).

Purchas (1998) suggested that a a^* value for bovine meat should ideally be between 18 and 22. As regards this factor, only unpressurized male meat or processed up to 200 MPa would fit the criterion, the remaining treatments considered as having faced "whitening" or being "pale", in spite of treatment 300 MPa at T1 presenting values very close to the minimum required for the ideal. These findings are contrary to those reported by Carlez et al. (1995), who observed no alterations in the a^* and b^* values of meat when subjected to pressure treatments up to 400 MPa, as well as the results reported by Marcos et al. (2010)

in their study of bovine meat from crossbred animals slaughtered at 24 months of age.

In the present research, HPP led to a decrease in b^* apart from the time element, which agrees with Cabral Neto et al. (2015). Furthermore, increases in yellow intensity were observed at pressures up to 200 MPa compared to non-pressurized meat, and the meat treated at pressures from 400 to 600 MPa was considered brown (Carlez et al., 1995). The authors attributed the reduction of b^* to pressure-induced formation of metmyoglobin, associated with iron oxidation in the Heme group of myoglobin (Carlez et al., 1995).

The HPP at 200 MPa resulted in the best level of tenderness based on instrumental texture analyses, with minimal impact on other quality parameters. Pressures above 200 MPa caused negative alterations in meat quality, making it pale and promoting lipid oxidation. Meat from younger females is more susceptible to color changes during pressurization due to its inherently lighter characteristic. HPP had a highly positive effect on whole male meat, leading to greater tenderness. The introduction of the Senepol breed in crossbreeding with Nelore animals allowed for a reduction in slaughtering age and broader meat cuts. Combined with HPP, this resulted in high-standard cuts that were considered premium due to their high tenderness. The potential of F1 Senepol/Nelore crossbreed meat subjected to HPP to produce high-value premium meat for particular markets will be further evaluated in sensory and consumer studies.

Authors' Contributions

Conceptualization: Cabral Neto O, Deliza R, Sousa SLG, Saldanha T, Gamallo OD, Santos WB, Rosenthal A. **Data curation:** Cabral Neto O, Saldanha T, Rosenthal A. **Formal analysis:** Cabral Neto O, Saldanha T, Rosenthal A. **Investigation:** Cabral Neto O, Deliza R, Saldanha T, Santos WB. **Methodology:** Cabral Neto O, Deliza R, Saldanha T, Santos WB. **Project administration:** Cabral Neto O. **Writing-review & editing:** Cabral Neto O, Santos WB

Conflict of interest

The authors declare that they have no potential conflicts of interest.

Data availability statement

The authors declare that the data present in the article offer open access to readers.

Declaration of use of AI technologies

The authors declare that they did not use any type of generative AI text.

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